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The effects of antenatal smoking and substance abuse on ventilatory responsiveness and chemoreceptor sensitivity in infants

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**The effects of antenatal smoking and substance abuse on
ventilatory responsiveness and chemoreceptor sensitivity
in infants**

Thesis submitted for the degree of

MD (research)

King's College London

University of London

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Abstract

Background: Infants of mothers who smoked (S) or substance misused (SM) during pregnancy have an increased risk of sudden infant death syndrome (SIDS).

Hypotheses: Infants of SM and S mothers will have poorer ventilatory responsiveness to hypercarbia and hypoxia and reduced chemoreceptor sensitivity compared to controls

Impairment of ventilatory responsiveness and chemoreceptor sensitivity will be greater in the infants of mothers who both substance abuse and smoke compared to those whose mothers only smoke

Methods: Three groups were recruited:

1. Infants of mothers with a history of substance misuse during pregnancy (SM infants)
2. Infants of mothers with a history of smoking during pregnancy (S infants).
3. Infants of mothers who neither smoked nor misused substances during pregnancy (Controls).

Ventilatory responses to hypercarbia and hypoxia were assessed in the newborn period and at 6-12 weeks of age

Results: In the newborn period both the SM and S infants had a lower ventilatory response to 2% and 4% CO₂ than the controls. The ventilatory response to CO₂ was lower in the SM infants compared to the S infants. In response to hypoxic challenge in the newborn period, SM infants had a greater magnitude of decline in minute volume than the S infants and the controls. In addition, the rate of decline in minute volume was greater in the SM infants and the S infants compared to the controls. At 6-12 weeks of age S and SM infants had a dampened ventilatory response to hypercarbia and a greater magnitude of decline in minute volume in response to hypoxia compared to controls. Dampening of the ventilatory response to hypercarbia was greater at the peak age of SIDS compared to the perinatal period.

Declaration

The statistical analysis for the neonatal ventilatory responses to hypercarbia and hypoxia described in Chapters 3 and 4 were carried out by Professor Janet Peacock; I thank her for her assistance in this regard. The department of biochemistry at King's College hospital carried out the laboratory analysis for cotinine and urine drug screen. Otherwise, all the work described in this thesis is my own.

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List of abbreviations

5-HT	5 Hydroxytryptamine
CEDIA	Cloned Enzyme Donor Immunoassay
CI	Confidence interval
cm	centimetres
CO ₂	Carbon Dioxide
CSE	Cigarette Smoking Exposure
ENGphr	Phrenic Electroneurograms
ETCO ₂	End Tidal Carbon Dioxide
g	gram
H ₂ O	Water
Hz	hertz
IFS	Infant Feeding Survey
L	litre
LBW	Low Birth Weight
LCD	Liquid Crystal Display
MIF	Mean Inspiratory Flow
min	Minute
ml	millilitres
MV	Minute Volume
NICHD	National Institute of Child Health and Human Development
NMDA	N-Methyl-D-Aspartate
NREM	Non Rapid Eye Movement
O ₂	Oxygen
PDGF	Platelet Derived Growth Factor
PH	Power of Hydrogen
PMA	Postmenstrual age
RCT	Randomised Controlled Trial
REM	Rapid Eye Movement
RR	Respiratory Rate
RR	Risk Ratio
RTN	Retrotrapezoid Nucleus
S	Smoking
s	second
SaO ₂	Oxygen Saturation
SATOD	Smoking At Time Of Delivery
SD	Standard Deviation
SIDS	Sudden Infant Death Syndrome
SM	Substance-Misuse
T _c	Time Constant

Ti	Inspiratory Time
TV	Tidal Volume
USA	United States of America

Publications arising from this thesis

1) Ali K, Rossor T, Bhat R, Wolff K, Hannam S, Rafferty GF, Peacock JL, Greenough A. Antenatal substance misuse and smoking and new-born hypoxic challenge response. Arch Dis Fetal Neonatal Ed-2016 Mar; 101(2): F143-8. doi: 10.1136/archdischild-2015

2) Ali K, Wolff K, Peacock JL, Hannam S, Rafferty GF, Bhat R, Greenough A. Ventilatory response to hypercarbia in newborns of smoking and substance-misusing mothers. Ann Am Thorac Soc. 2014 Jul; 11(6): 933-8. doi: 10.1513/AnnalsATS.201403-124OC.

3) Ali K, Rossor T, Bhat R, Wolff K, Hannam S, Rafferty GF, Greenough A. Ventilatory responses to Hypercarbia in Infants of Mothers Who Smoke and Misuse Substances. 2016 JPediatr May 20. pii: S0022-3476 (16) 30188-3. doi: 10.1016/j.jpeds.2016.04.078.

4) Ali K, Rossor T, Bhat R, Wolff K, Hannam S, Rafferty GF, Greenough A. Responses to hypoxia in infants of smoking and substance-abusing mothers at the peak age for SIDS. Pediatr Pulmonology 2016 Oct 10. doi: 10.1002/ppul.23620

1 Introduction

1.1 Background

1.1.1 Historical perspective of Sudden Infant Death (SIDS)

The sudden, tragic death of sleeping babies has been a feature of history for over 2000 years. "Overlaying" or the accidental suffocation of an infant by an adult who rolled on the baby while sleeping was, for centuries, thought to be the only reasonable explanation for an apparently healthy infant going peacefully to sleep and never waking up again. In ancient Egypt, a mother who was judged to be responsible for doing this was sentenced to hold the dead infant for three days and nights. The first known medical textbook written during the second century A.D. by the Greek physician Soranus of Ephesus instructs mothers and wet-nurses never to sleep with infants in case they should accidentally fall asleep on the baby and somehow suffocate them. References to "overlaying" existed throughout the centuries. In 1291 in Germany there was a dictum-forbidding mothers to take children under 3 years of age into their beds. In the 14th century in England, overlaying of one's infant was considered a sin and punishments by the church were handed to mothers if overlaying occurred. Later on during the 16th century in Italy, penalties were given to unwed mothers whose infants suffocated in bed. The "Arcuccio", a wooden framework with arches of iron was described in "The Art of Nursing", London in 1733. It was placed between the husband and wife to prevent the child being overlaid (1895). In the 19th century, there was a transition of punishment for infanticide from ecclesiastical to secular authority with civil and coroner's courts beginning to investigate cases of overlaying.

A more humanitarian attitude towards mothers whose infant had died suddenly and the mother would have faced prosecution followed the thymic theory by Morgagni in 1773.

He suggested that the gland impinged on the trachea cutting off the airway or the blood supply to the head resulting in adverse stimulation of nerves controlling respiration and thereby causing suffocation.. The thymic theory also contributed to the ill-conceived concept of thymic irradiation in United States from 1926-1947. Nineteenth-century doctors naturally tried to explain scientifically the sudden deaths of babies, and one of the first such explanations was that the infant suffered from some sort of respiratory event. By the beginning of the twentieth century, sleep apnea was considered a cause. By the 1930s, the role of infection was being considered and by the 1940s, most American mothers were no longer taking their children to bed with them for fear of accidentally smothering them.

1.1.2 Definition of Sudden Infant Death Syndrome (SIDS)

Sudden Infant Death (SIDS) was first described over six decades ago. The original emphasis was on clinical history alone. Werne and Garrow in 1953 referred to SIDS as ‘sudden apparently unexplained death during infancy’. (Garrow and Werne, 1953, Werne and Garrow, 1953) Adelson and Kinney in 1956 reported their cases as ‘sudden and unexpected death in infancy and childhood’.(Adelson and Kinney, 1956). They noted that these deaths occurred in ‘a child who was thought to be in good health or whose terminal illness appeared to be so mild that the possibility of a fatal outcome was not anticipated’. The definition of SIDS has undergone considerable changes since its original description. The requirement for autopsy was first emphasised by Beckwith in 1973: ‘the sudden death of any infant or young child which is unexpected by history and in which a thorough post-mortem examination fails to demonstrate an adequate cause of death (Beckwith, 1973). In 1991 that definition was revised by the National Institute of Child

Health and Human Development, restricting the cases to less than one year of age, and including examination of the death scene as part of the definition: ‘The sudden death of an infant under one year of age which remains unexplained after a thorough case investigation, including performance of a complete autopsy, examination of the death scene, and review of the clinical history’ (Willinger et al., 1991)

The most recent revision of the definition of SIDS occurred in 2004, often referred to as the San Diego definition, which incorporated the apparent association with sleep and emphasised evaluating the circumstances of death: ‘the sudden unexpected death of an infant less than one year of age, with onset of the fatal episode apparently occurring during sleep, that remains unexplained after a thorough investigation, including performance of a complete autopsy and review of the circumstances of death and the clinical history’ (Krous et al., 2004)

1.1.3 Incidence of Sudden Infant Death Syndrome (SIDS)

Sudden Infant Death remains the leading cause of death in the post neonatal period in the developed world (Blair et al., 2006b). Since the ‘Back to Sleep’ campaign in 1991, the number of SIDS deaths has fallen by 75% in England and Wales (Health Statistics Quarterly winter 2004. Office for National Statistics-The Stationery Office). The SIDS rate in England and Wales was 1.4 deaths per 1000 live births in 1991. In 2012, there were 221 deaths from SIDS in England and Wales. This is the equivalent of 0.3 deaths per 1,000 live births and accounted for 8% of all infant deaths occurring in 2012. Eight out of ten unexplained infant deaths occurred in the post-neonatal period (between 28 days and one year) and 64% were boys. The rate of unexplained infant death was three times higher among low birthweight

babies (less than 2,500g) than babies with a normal birthweight (2,500g and over). (Unexplained Death in Infancy: England and Wales 2012- Office for National Statistics).

Among the industrialized nations, Japan has the lowest reported SIDS rate (0.09 case per 1000 infants), New Zealand has the highest rate (0.80 per 1000), and the United States has an intermediate rate (0.57 per 1000) (Moon et al., 2007a) . A striking discrepancy exists among racial and ethnic groups that have been studied; with SIDS rates that are two to seven times the national averages among native Americans (Iyasu et al., 2002) and blacks (Hauck et al., 2003) in the United States; among Maoris in New Zealand (Mitchell et al., 1994) and among aboriginal Australians (Ford and Nelson, 1995) . SIDS data from 13 predominantly industrialized countries, (Hauck and Tanabe, 2008) showed that the majority of countries had a major decrease in SIDS rates from 1990 to 2005 , with the largest decreases occurring before 2000 (Hauck and Tanabe, 2008). These decreases ranged from 40% in Argentina to 83% in Ireland.

1.1.4 Pathophysiology of SIDS

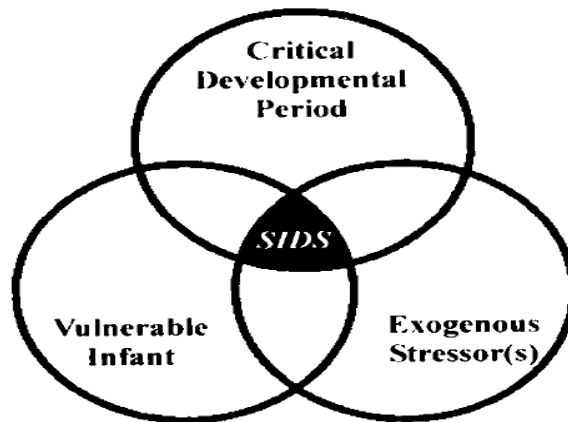


Figure 1.1 Triple Risk Model (Filiano and Kinney, 1994)

The precise pathophysiology of SIDS is not clearly defined, but is widely thought to be multifactorial. The risk model was first proposed by Froggatt et al. in 1971: ‘The age range represents a period of enhanced physiological vulnerability in which some critical combination of extrinsic (e.g., infection and sleep) and intrinsic (not yet unequivocally identified) factors can prove lethal’ (Froggatt et al., 1971).

The fatal triangle was proposed in 1993 by Rognum and Saugstad (Rognum and Saugstad, 1993). They proposed the interplay of three factors: (i) a vulnerable phase in the development of the central nervous system and the immune system in the first months after birth, (ii) predisposing factors, such as brainstem astrogliosis or genetic make-up, and (iii) a trigger event such as overstimulation of the immune system.

The ‘Triple Risk Model’ describes SIDS as an event that results from the intersection of three overlapping factors: (i) a vulnerable infant, (ii) a critical development period in homeostatic

control, and (iii) an exogenous stressor (Filiano and Kinney, 1994) (Figure 1.1). The vulnerability of the infant (e.g. premature birth) lies latent until the infant enters a critical period of development (usually one to six months) and is exposed to an exogenous stressor (e.g. prone sleeping or hypercarbia).

Studies have shown that some SIDS victims have a subtle underlying immaturity or abnormality of the central nervous system (CNS) making them vulnerable to various physiological challenges, including being positioned prone. Filiano & Kinney (Filiano and Kinney, 1992a) showed evidence of arcuate nucleus hypoplasia in SIDS victims. This is an area thought to be an integrative site for chemosensitivity, ventilatory control, autonomic function and arousal (Filiano and Kinney, 1992a). Kinney's group postulated that SIDS is caused by an underlying brainstem abnormality in neural networks that mediate protective responses to asphyxia (Kinney et al., 2009). They have reported deficiencies related to the neurotransmitters serotonin and γ -aminobutyric acid in infants who died of SIDS (Kinney and Thach, 2009). A reduction of serotonin receptor binding has also been found in SIDS victims in four other regions of the ventral medulla believed to be involved in the homeostatic response (Panigrahy et al., 2000).

Kinney and Thach postulated the following lethal sequence in the pathophysiology of SIDS: rebreathing in face-down position, face-covered in supine position or obstructive apnea occurring during sleep being life-threatening event that causes severe asphyxia, brain hypoperfusion or both (Kinney and Thach, 2009). The vulnerable infant does not arouse and turn his head in response to this asphyxia resulting in rebreathing or inability to recover from apnea. Progressive asphyxia leads to loss of consciousness and rapid-onset hypoxic-coma. Extreme bradycardia and hypoxic gasping begins. Autoresuscitation fails with onset of ineffectual gasping resulting in death. Subsequently Randall et al. have compared SIDS

cases in circumstances consistent with asphyxia and infants dying suddenly without obvious asphyxia-generating circumstances. There were, however, no differences in the mean neurobiochemical parameters (Randall et al., 2013). Brain-stem abnormalities were associated with both asphyxia-generating and non-asphyxia-generating conditions. They concluded that safe sleep messages are essential for all infants

1.1.5 Risk factors of SIDS

Risk factors for SIDS can be divided into extrinsic and intrinsic categories (Paterson et al., 2006). Extrinsic risk factors are physical stressors that would place a vulnerable infant at risk for asphyxia or other homeostatic derangement. Such extrinsic factors include prone and side-sleeping positions, bedclothes that cover the head, sleeping on sofas or other soft furniture in which the infant could become wedged, a high ambient temperature in the sleeping environment, soft bedding, and bed sharing (Mitchell et al., 1994, Willinger et al., 1994) (Iyasu et al., 2002) (Hauck et al., 2003) (Kemp et al., 2000) (Ponsonby et al., 1993).

Although the incidence of a prone sleeping position is currently 20% or less (Hauck and Tanabe, 2008) (Corwin et al., 2003), 30 to 50% of infants with SIDS are still found in the prone position (Mitchell et al., 2008). Approximately 50% of sudden infant deaths occur when infants are sharing a bed, sofa, or sofa chair with another person (Hauck et al., 2003). The prone sleeping position and a soft mattress are associated with an increase by a factor of 20 in the risk of SIDS, suggesting the additive risk for these two factors (Ponsonby et al., 1993). However, there are arguments in favour of bed sharing, which include facilitation of breast-feeding and nighttime bonding, behaviours that are beneficial to an infant's wellbeing (McKenna et al., 2007). Approximately 10% of SIDS cases occur in infants who sleep in a supine position and do not share a bed and whose face is not covered by bedclothes (Beal,

2000). This finding reinforces the points that such risk factors are not causative and that the causes of SIDS are multifactorial.

In addition to extrinsic risk factors related to external events around the time of death, intrinsic factors are postulated to affect the underlying vulnerability of the infant to SIDS. Intrinsic risk factors can be subdivided into developmental factors, such as prematurity (Horne, 2006), and genetic factors, such as familial SIDS (i.e., a recurrence of SIDS in subsequent siblings) (Oyen et al., 1996, Hunt, 2001) , male sex (by a 2:1 ratio), and race or ethnic group (Hauck et al., 2003).

Certain genetic polymorphisms have been found to increase the risk of SIDS (Opdal and Rognum, 2004, Hunt, 2001) (Weese-Mayer et al., 2007) (Hunt, 2005). Polymorphisms associated with SIDS have been reported in a variety of genes involved in autonomic function, neurotransmission, energy metabolism, and response to infection (Hunt, 2001) (Weese-Mayer et al., 2007) (Hunt, 2005). In addition, the vulnerable infant's response to environmental factors may actually reflect aberrant intrinsic responses. For that reason, events and environmental conditions extrinsic to the infant, such as poverty (Beal, 2000) (Ford and Nelson, 1995) (Malloy and Hoffman, 1995) (Blair et al., 2006a), adverse prenatal exposures to certain substances (e.g., cigarette smoke and alcohol or illicit drugs) (Moon et al., 2007a) (Iyasu et al., 2002) (Blair et al., 2006b) (Blair et al., 1996) and postnatal exposure to cigarette smoke, may trigger intrinsic responses in the vulnerable infant. For instance, prenatal exposures to alcohol and cigarette smoke have a direct effect on neurotransmitter systems that are critical to homeostatic control in the developing human brain (Duncan et al., 2008).

Table 1.1 Risk factors associated with an increased risk of SIDS

<ul style="list-style-type: none"> • Male gender • Ethnicity • Sociodemographic factors: <ul style="list-style-type: none"> Low maternal age and education High birth order, Single motherhood, Low maternal & paternal education Maternal and paternal unemployment • Prematurity and low birth weight • Substance misuse • Smoking • Sleep environment & practices <ul style="list-style-type: none"> Use of Pillows and soft bedding- • Overheating • Prone positioning • Lack of prenatal care • Genetic predisposition
--

Recently, changes have been identified in the demographic characteristics of infants who succumb to SIDS. In the United Kingdom, for example, the proportion of SIDS deaths occurring in term infants has decreased, whereas the proportion in preterm infants has increased from 12% to 34% ($P < 0.001$) (Blair et al., 2006b). Furthermore, the proportion of SIDS deaths occurring in families living in poverty has increased from 47% to 74% ($P = 0.003$), and the proportion of SIDS deaths in infants of mothers who smoked during pregnancy has increased from 57% to 87% ($P = 0.004$) (Blair et al., 2006b).

The delineation of risk factors has been crucial in establishing the biologic basis of disparities in the incidence of SIDS among racial and ethnic groups (Iyasu et al., 2002, Hauck et al., 2003, Mitchell et al., 1992). Although high SIDS rates among American blacks and native

Americans, Maoris, and aboriginal Australians may reflect factors that are independent of economic levels, poverty is associated with higher rates of maternal alcohol use and smoking during pregnancy, prematurity and bed sharing (due to a lack of space and funds for cribs) (Mitchell et al., 1992) (Iyasu et al., 2002, Hauck and Tanabe, 2008).

1.1.6 Age at death from SIDS

SIDS deaths tend to occur under six months of age with a peak incidence between two and three months of age (Peterson et al., 1979); (MacArthur and Bartholomew, 1987); (Adams et al., 1990). It has been noted that racial origin, birth weight and maternal smoking have an effect on the mean age at death. Black infants were noted to die at a younger age compared to non-black infants in the NICHD study (Hoffman and Hillman, 1992). Very low birth weight infants died at six weeks older postnatal age in the California Birth Defects Monitoring Program (Grether and Schulman, 1989). Others, however, have demonstrated that prematurely born infants die of SIDS at a 4 to 6 weeks younger compared to the term infants (Malloy and Hoffman, 1995). Infants of smoking mothers died at a younger postnatal age in the Swedish cohort, the incidence of SIDS in the infants born to smoking mothers peaked at five to eight weeks compared to the peak age of nine to thirteen weeks in infants of non-smoking mothers (Haglund and Cnattingius, 1990). These facts emphasise that premature infants and infants born to smoking mothers are particularly vulnerable to SIDS and die at a younger age.

1.2 Smoking and Sudden Infant Death

1.2.1 Prevalence of smoking during pregnancy

To estimate the prevalence of maternal smoking during pregnancy, self-reported data from birth registers were often used due to their accessibility (Mattsson et al., 2016). Self-reporting, however, carries a risk of underreporting due to the stigma surrounding smoking in pregnancy, leading to biases. Indeed, a reliable measure of smoking exposure is key for planning future studies concerning health effects of tobacco use (England et al., 2007)

Cotinine (the primary metabolite of nicotine), passes readily through the placenta and can be measured both in maternal and umbilical cord serum. Umbilical cord cotinine is considered the gold standard of measuring fetal tobacco exposure (Pichini et al., 2000). Studies looking at the level of agreement between self-reporting of smoking and biochemical screening for smoking during pregnancy have provided conflicting results. Although some have found a correlation between self-reported smoking and biomarker measures (George et al., 2006, Kvalvik et al., 2012), others have demonstrated a significant underreporting using the self-reporting method as opposed to biochemical screening (Lindqvist et al., 2002, Webb et al., 2003, Parna et al., 2005). A validation study comparing self-reports with serum cotinine of a national sample found that 23% of pregnant smokers and 10% of non-pregnant smokers did not disclose their smoking habits (Dietz et al., 2011). A study from Sweden found that the agreement between mothers' self-reported smoking habits during pregnancy and their levels of serum cotinine was high, as was the transfer of cotinine from mother to fetus (Mattsson et al., 2016)

Smoking At Time Of Delivery (SATOD) dataset and the Infant Feeding Survey (IFS) are the two main data sources that provide information on the prevalence of smoking during pregnancy in England (Health and Social Care Information Centre). SATOD data have been

collected from primary care trusts quarterly by the Health and Social Care Information Centre. In order to collect these data, health professionals in maternity units are required to record the smoking status of every woman at the time of delivery. The IFS is a UK wide retrospective survey that is undertaken every five years and includes information on self-reported maternal smoking behaviour, involving a sample of women who have registered live births during a particular time period. Both of these data sources showed that reported rates of smoking in pregnancy in England have fallen over time. The most recent SATOD dataset showed that 11.4 per cent of mothers were recorded as smokers at the time of delivery for 2014/15, which is lower than 2013/14 (12.0%) and continues the steady year-on-year decline in the percentage of women smoking at the time of delivery from 15.1 per cent in 2006/07 (Statistics on Women's Smoking Status at Time of Delivery: England, Quarter 4, April 2014 to March 2015 - <http://www.hscic.gov.uk/>). The IFS data in 2010 reflects similar results. Around a quarter of mothers (26%) in the UK smoked in the 12 months before or during their pregnancy, which was reduced from 33% in 2005. Of mothers who smoked before or during their pregnancy, over half (54%) gave up at some point before the birth (Infant Feeding Survey 2010-The Information Centre for Health & Social Care <http://www.hscic.gov.uk/>). Twelve per cent of mothers continued to smoke throughout their pregnancy, down from 17% in 2005. This translates into over 83,000 infants born to smoking mothers each year. In some areas of England these rates are much higher. For example, in the North East, 20% of women were recorded as smoking at the time of delivery (Infant Feeding Survey 2010-The Information Centre for Health & Social Care <http://www.hscic.gov.uk/>). The 2010 IFS also showed that smoking rates not only vary by region but also by age and social group. Pregnant women from unskilled occupation groups are five times more likely to smoke than professionals, and teenagers in England are six times more likely to smoke than older mothers. Although both sources show that smoking prevalence among pregnant women is

falling, it is important to note that they are both likely to underestimate the true number of pregnant smokers in England. This is due to women under-reporting smoking behaviour (Shipton et al., 2009) and incomplete or incorrect completion of data collection forms. In addition to this, the prevalence may be under-estimated further due to the fact that women who miscarry, or have a stillbirth, are not included in the data sets; missing an important category of women where smoking may have contributed to their pregnancy loss.

In the United States of America, the Pregnancy Risk Assessment Monitoring System (PRAMS) provided data on smoking before, during, and after pregnancy from 40 sites during 2000–2010 (Tong et al., 2013). There were moderate, but significant decreases in the prevalence of women smoking while pregnant (from 13.3% to 12.3%) and after delivery (from 18.6% to 17.2%) in a subgroup of sites. Several sites had significant reductions in smoking prevalence around the time of pregnancy. During 2000–2010, in three sites, the prevalence decreased for smoking before, during, and after pregnancy, and in eight sites, the prevalence decreased for one or two of the measures (before, during, or after pregnancy). A comparable decline in maternal smoking was also reported in national data from Sweden and Denmark (Cnattingius, 2004).

1.2.2 SIDS and Maternal Smoking

Since the “Back to Sleep” campaign, cigarette smoke exposure is the leading independent risk factor for the occurrence of SIDS (Mitchell et al., 1997, Moon et al., 2007b). The first report of the association of maternal smoking and SIDS was published by Steele et al (Steele and Langworth, 1966). An odds ratio of 2.4 was obtained which was not significantly reduced by allowance for low birth weight (Steele and Langworth, 1966). This was followed

by a series of case- control and cohort studies showing an increased risk of SIDS in relation to both prenatal and postnatal maternal smoking. Schoendorf and colleagues showed that both in-utero and ex-utero passive exposure to smoking increased the risk of SIDS by two to three fold (Schoendorf and Kiely, 1992). According to both the National Institute of Child and Human Development study (Hoffman et al., 1988) and a prospective Australian study in Tasmania, (McGlashan, 1989) maternal smoking was more common amongst mothers of SIDS victims (OR=3.8, and OR =3.0, respectively). A dose dependent effect of exposure to tobacco smoking and the risk of SIDS was demonstrated in the New Zealand cot death study that included 162 SIDS deaths (Mitchell et al., 1991). The odds ratio was 1.87 when exposure was less than 10 cigarettes per day, which increased to 2.64 and 5.06 when infants were exposed to 10-19 cigarettes and more than 20 cigarettes per day respectively (Mitchell et al., 1991). A case control study undertaken in Scotland showed that exposure to cigarette smoke increased the SIDS risk much more if the mother or both parents smoked as compared to paternal smoking alone or if neither parent smoked in the household (Brooke et al., 1997). Similarly, the risk of SIDS in mothers who smoked in pregnancy was significantly higher (OR 4.1; 95% CI 3-5.7) in the Nordic epidemiologic SIDS study (Oyen et al., 1997). MacDorman and colleagues (MacDorman et al., 1997) compared the association between SIDS and smoking between Sweden and United States and concluded that the strong association between SIDS and smoking persisted after adjustment for maternal age, birth order and infant birth weight. They also demonstrated a dose-response relationship with the odds ratios increasing from 1.6 to 2.5 for mothers who smoked between 1 and 9 cigarettes per day during pregnancy to 2.3 to 3.8 for mothers who smoked more than 10 cigarettes per day (MacDorman et al., 1997). In a population based case control study involving three regions of the United Kingdom, maternal smoking increased the risk of SIDS (OR 2.1). A combination of paternal smoking/ postnatal exposure to an in-utero maternal smoking further

increased the risk of SIDS (OR 2.5 and 2.9 respectively) (Blair et al., 1996). The first systematic review on the association of SIDS and maternal smoking was published by Anderson and Cook in 1997. The review included 39 case control and cohort studies (Anderson and Cook, 1997). The unadjusted pooled odds ratio for prenatal maternal smoking was 2.77 (95% CI 2.45 to 3.13). After adjustment for a variety of confounders the pooled odds ratio was reduced to 2.08 (95% CI 1.83 to 2.38) and was similar in cohort and case-control studies. The OR for postnatal smoking after controlling for prenatal maternal smoking was 1.94 (95% CI 1.55 to 2.43).

A study from Sweden utilizing the Swedish Medical Birth Registry following the back to sleep intervention campaign showed that the adjusted OR for maternal smoking and SIDS was 4.11 (95% CI: 2.72, 6.21) (Chong et al., 2004). In a retrospective cohort study utilizing the Colorado birth registry over the time period of the Back to Sleep campaign, “smoking” exposed infants were 1.9 times (95% CI 1.6 to 2.3) more likely to die of SIDS (Anderson et al., 2005). In a study from the Netherlands with 142 cases of SIDS that occurred after the parental education on sleeping position and 2,841 controls, postnatal smoking was identified as an important risk factor for SIDS (OR one parent=2.5 [1.2, 5.0]; both parents=5.77 [2.2, 15.5]; maternal=2.7 [1.0, 6.4]; paternal=2.4 [1.3, 4.5] (Liebrechts-Akkerman et al., 2011). A recent meta-analysis by (Zhang and Wang, 2013) of 35 case control studies on the association between maternal smoking and SIDS risk between 1990 to 2011 provided similar results. Both prenatal and postnatal maternal smoking, were associated with a significantly increased risk of SIDS (OR = 2.25, 95% CI = 2.03–2.50 for prenatal maternal smoking, and OR = 1.97, 95% CI = 1.77–2.19 for postnatal maternal smoking analysis, respectively). Infants co-sleeping with smoking mothers also had a significantly increased risk of SIDS (OR = 1.86, 95% CI = 1.28–2.69) (Zhang and Wang, 2013)

A number of studies have reported that maternal smoking modifies the effect of some risk factors for SIDS. A study from Germany found that preterm birth, low birthweight and low number of antenatal visits did not increase the risk of SIDS among infants of maternal non-smokers, but were important risk factors for infants of maternal smokers (Schellscheidt et al., 1997). For example, compared with normal birthweight (2500+ g) infants, low birthweight (<2500 g) was associated with an increased risk of SIDS in infants of mothers who smoked (RR = 2.62), but not in infants of non-smoking mothers (RR = 1.17). Alternatively, the relative risk of SIDS associated with maternal smoking was 7.96 for infants less than 2500 g and 3.56 for infants more than 2500 g. Meta-analysis of six studies showed that bed sharing was a major risk factor for SIDS if the mother was a smoker (OR = 2.06, 95% CI = 1.70, 2.50), but was associated with only a slightly increased risk if the mother was a non-smoker (OR = 1.42, 95% CI = 1.12, 1.79) (Scragg and Mitchell, 1998).

1.2.3 Pathophysiology of SIDS associated with smoking

Prenatal smoking may cause morphological placental changes that lead to chronic fetal hypoxic stress and abnormal lung and brain development (Hofhuis et al., 2003). Nicotine is a powerful stimulant of cholinergic neurons and has been postulated to act upon nicotinic acetylcholine receptors either centrally (Kinney et al., 1993) or peripherally (Holgert et al., 1995). Postnatal exposure to cigarette smoke may trigger intrinsic responses in the vulnerable infant and have a direct effect on neurotransmitter systems that are critical to homeostatic control in the developing human brain (Kinney and Thach, 2009). Measurements of nicotine and its metabolites in babies diagnosed as dying from SIDS indicate that significant exposure has occurred around the time of death (Milerad et al., 1994, Milerad et al., 1998). High levels of cotinine in the pericardial fluid of SIDS victims who were co-sleeping and had focal organ

lesions in the liver and the heart could reflect previous hypoxic ischemic insult (Rajs et al., 1997). Delayed myelination of the central nervous system particularly in areas of somatomotor and limbic-paralimbic regions has been seen in SIDS cases suggesting a central nervous system insult may contribute to SIDS (Kinney et al., 1991). Studies investigating the association between smoking and the development of SIDS have highlighted similar abnormalities in the central nervous system. Storm et al conducted a pathological examination of the brainstem of SIDS victims and showed gliosis of the inferior olivary nucleus in infants of mothers who smoked; the degree of gliosis correlated significantly with the number of cigarettes smoked during pregnancy (Storm et al., 1999).

High levels of cotinine in the fetus are associated with effects on both cell replication and programmed cell death (apoptosis) (Slotkin et al., 1997a). The neuronal injury in the brain stem found in many SIDS victims has consequently been ascribed to tobacco smoke exposure (Krous et al., 1981), although it is not clear whether the neuronal damage is a primary toxic effect of substances in tobacco smoke or secondary to hypoxic or ischemic episodes that may be associated with smoking exposure (Rognum and Saugstad, 1991).

Nicotine stimulates cholinergic neurons and the release of dopamine and noradrenaline from synaptic nerve terminals in the brain and elsewhere (Benowitz, 1990) Dopaminergic neurons are predominantly involved with regulation of neuroendocrine functions, locomotion and reward-seeking behavior (Goldman-Rakic, 1998) (Schultz, 1997) (Wooten, 1997). Dopamine mediated functions adversely changed by fetal nicotine exposure include hyperactivity (Richardson and Tizabi, 1994) and disturbances in cortisone and pituitary hormones release (Fuxe et al., 1989). Dopamine concentration was decreased in the ventral tegmental area and the striatum in offspring of nicotine-treated dams, timed-pregnant Sprague-Dawley rats

(Richardson and Tizabi, 1994). Noradrenergic neurons regulate a variety of autonomic functions including responses to stress (Esler et al., 1995, Page and Valentino, 1994, McCormick et al., 1991). Noradrenaline mediated functions adversely affected by nicotine include a decreased ability for sympathetic activation in response to exogenous stress (Page and Valentino, 1994).

1.2.4 Cardiopulmonary effects of exposure to maternal smoking

Two possible explanations for the increased risk of SIDS in mothers who smoked during pregnancy are a reduced ability to recover from prolonged apnea or insensitivity to hypoxia or hypercarbia. There is evidence from both humans and animals studies that exposure to tobacco smoke or nicotine during pregnancy affects fetal development of the brainstem where the main cardiorespiratory centers are localized (Slotkin et al., 2005) (Lavezzi et al., 2005) (Slotkin et al., 2011). Prenatal cigarette smoke exposure (CSE) results in apoptotic cell death in the brainstem regions that control respiratory activity (Nie et al., 2013).

Although some studies in human infants observed no significant difference in respiratory drive between the infants of smokers and non-smokers (Schuen et al., 1997) (Poole et al., 2000) (Campbell et al., 2001). Others showed that 2- 24 months old infants born to smoking mothers have a reduced drive to breath during normoxia and a diminished ventilatory response to hypoxia (Ueda et al., 1999). Moreover, infants of mothers who smoked antenatally prior to maternity unit discharge (ie, before passive postnatal smoke exposure) had a dampened ventilatory response to hypercarbia when compared to controls. This meant that the time constant of the infant's response in increasing their minute ventilation to compensate for the added dead space was longer (Bhat et al., 2005). Hypercarbia is the most

important stimulus to ventilation with added dead space; therefore, those results are compatible with dampened chemoreceptor function in infants exposed to cigarette smoking in utero (Bhat et al., 2005). In contrast to those observations, another study found that ventilatory responses to hypoxia and hypercapnia were not different between 2-3 months aged infants of smoking mothers and controls. It was noted however, that infants of mothers who smoked during pregnancy had a deficient arousal response to hypoxia (Lewis and Bosque, 1995).

There is also conflicting evidence from animal studies. For example, studies in neonatal rats in vivo indicated that no significant effect of prenatal nicotine exposure was found on ventilatory responses to moderate hypoxia or hypercapnia (Bamford et al., 1996) (Poole et al., 2000). However, another study showed that the ventilatory response to hypoxia was significantly attenuated during quiet sleep in nicotine-exposed neonatal lambs (Hafstrom et al., 2002). It was also found that continuous infusion of nicotine to pregnant rats from day six of gestation to days five or six postpartum resulted in an impairment in the ability of the newborn pups to auto-resuscitate from primary apnea during repeated exposure to hypoxia (Fewell and Smith, 1998). In addition, rat pups exposed to nicotine during fetal life exhibited deficient adrenomedullary catecholamine release in response to hypoxia. As a result, the rats died at the same hypoxic levels that unexposed rat pups survived without major ill effects (Slotkin et al., 1995). The pups also lacked the normal increase in heart rate that should occur in response to hypoxia (Slotkin et al., 1997b). Infusion of nicotine in lambs at mean ages of 7, 17, and 27 days resulted in attenuation of the ventilatory response to hypoxia and augmentation of the response to hyperoxia, suggesting that exposure to nicotine altered peripheral chemoreceptor oxygen sensitivity and it may have also affected the central processing of the chemoreceptor input (Milerad et al., 1995). Data from animal studies

suggest that nicotine interferes with the postnatal resetting of oxygen sensitivity of the peripheral arterial chemoreceptors by increasing carotid body tyrosine hydroxylase mRNA and dopamine release (Holgert et al., 1995). Similarly, prenatal Cigarette Smoking Exposure (CSE) impaired responses to hypoxia in vitro in medullary slice preparations from neonatal rats (Nie et al., 2013). Studies in neonatal mice medullary slices indicated that prenatal nicotine exposure switches cholinergic mechanisms of central chemosensory responses from muscarinic receptors to nicotinic receptors. Modification of the cholinergic contribution to central chemoreception may produce respiratory dysfunctions (Coddou et al., 2009). Indeed, nicotine is only one of the toxic components of cigarette smoke and many other ingredients can cross the placenta barrier to reach the fetus such as carbon monoxide. Carbon monoxide from cigarette smoke can cross the placenta barrier and inhibit the release of oxygen into fetal tissues (Longo, 1976). Recurrent intrauterine hypoxic insults affect the development of the fetal central nervous structure, where the retrotrapezoid nucleus (RTN) the most important central chemoreceptor, is localized (Guyenet et al., 2008)

Serotonin (5 hydroxytryptamine, 5-HT) neurons play a key role central chemoreception (Richerson, 2004). There is now emerging evidence that prenatal exposure to alcohol and cigarette smoke is associated with harmful effects on the development of the human medullary 5-HT system (Kinney, 2009). Exposure to maternal smoking, alcohol and cocaine also causes upregulation of the 5 HT transporter (5HTT) which is the key regulator of the 5HT levels at the synapse (Awtry and Werling, 2003),(Bauman et al., 2000), (Kelai et al., 2003). There is recent evidence from animal studies in mice that prenatal-perinatal exposure to nicotine alter the activity, electrical properties and central chemosensitivity of the raphe obscurus neurones (Cerpa et al., 2015).

Another tentative mechanism by which smoking increases the risk of SIDS is an increased risk of respiratory infections in infancy. Infants who live in smoking environment have more frequent upper airway infections than infants in non-smoking homes (Taylor and Wadsworth, 1987) Nicotine may enhance adverse immunological reactions to trivial bacterial infections and induces fatal outcomes in chick embryos (Sayers et al., 1995). The proposed mechanism may be an immunological potentiation of the antigenic properties of staphylococcal toxins by nicotine (Blackwell et al., 1994).

Prenatal Cigarette Smoking Exposure (CSE), has been shown to affect lung volume (Tager et al., 1995) (Hanrahan et al., 1992) and passive lung mechanics (compliance and resistance) (Hanrahan et al., 1996). Another study in newborns of smoking mothers at median age of 2.7 days showed that maternal smoking affected tidal flow-volume ratios and compliance of the respiratory system in girls, independently of the reduced body size (Lodrup Carlsen et al., 1997). The mechanism by which maternal smoking induces the reduction in lung size has not been specifically elucidated. One important factor is that smoking decreases fetal breathing and the decrease in rhythmic mechanical distension of airways by fetal breathing movements hampers normal lung growth (Moessinger et al., 1990).

Since the majority of these changes are already present at birth they are not likely to be produced by the irritant effect of tobacco smoke but are rather a consequence of a suboptimal fetal organ growth (Mitchell and Milerad, 2006), (Milner et al., 2007).

It is also important to determine whether antenatal smoking affects chemoreceptor responses to hypercarbia and hypoxia both in the perinatal period and at the peak age for SIDS, which is an important aim of this thesis

1.2.5 Cotinine

Cotinine, the major proximate metabolite of nicotine, is widely used as a biomarker of tobacco exposure (Benowitz, 1996, Benowitz et al., 2009b). It is produced by the metabolism of nicotine in the liver by cytochrome P 450 which converts almost 75% of the nicotine to cotinine (Hukkanen et al., 2005). Cotinine passes readily through the placenta and can be measured both in maternal and umbilical cord serum. Umbilical cord cotinine is considered the gold standard of measuring fetal tobacco exposure.(Pichini et al., 2000). Cotinine's half-life is longer (12-36 h) than nicotine's (2 h) (Kyerematen and Vesell, 1991, Pilotti, 1980). Urine cotinine concentrations average fourfold to six-fold higher than those in blood or saliva, making urine a more sensitive matrix to detect low-concentration exposure (Benowitz et al., 2009a). Indeed, cotinine provides a specific and objective assessment of the individual's actual smoking exposure to allow assessment of exposure-response relationships. Several studies used levels of cotinine and other biochemical metabolites of nicotine to both validate self-reported smoking practices during pregnancy (Eliopoulos et al., 1994) (Haley et al., 1983) and predict pregnancy outcomes such as birth-weight (Haddow et al., 1987, Haddow et al., 1988, English et al., 1994). In this thesis, therefore, maternal smoking will be assessed not only by self-report but by analysis of infant and maternal urinary cotinine levels

1.3 Maternal substance abuse and SIDS:

1.3.1 Prevalence of substance misuse during pregnancy

A survey carried out by the Office for National Statistics on behalf of the Department of Health showed that approximately one in a thousand women in Great Britain is dependent on opioids; the majority of these women were of child bearing age (Psychiatric Morbidity Amongst Adults Living in Private Households. London: Office for National Statistics, The Stationary Office; 2001.) Anonymous urine testing in pregnant mothers in the East of London found that 10.6% of urine samples were positive for illicit drugs (Farkas et al., 1995) A similar study in South London highlighted that 16% of women at pregnancy booking had evidence of substance misuse on the basis of urine toxicology screening,(Sherwood et al., 1999) suggesting that one in six women in South London were misusing drugs in early pregnancy. A survey of English and Welsh maternity units in 1993 estimated the number of babies born to substance-abusing mothers was 0.81 per 1000 deliveries (Patni et al., 2008)

A higher prevalence of maternal substance misuse has been reported from some, but not all, population-based studies in the United States. Ostrea and colleagues reported a 31% prevalence of cocaine use during pregnancy based on toxicological screening of meconium at birth; 44% of 3010 babies tested positive for opiates, cocaine, or cannabis (Ostrea et al., 1992). A study in Boston, in which cocaine use was assessed by interviews during pregnancy and urine samples obtained prenatally and immediately postpartum demonstrated that 28% of urine samples tested positive for marijuana and 17% for cocaine or its metabolites (Frank et al., 1988). Of the cocaine users, 24% denied use at the time of the interview and were identified solely by urine assay(Frank et al., 1988). A study from Florida, however, reported

only a 14.5% prevalence of maternal substance misuse during pregnancy (Chasnoff et al., 1990).

Maternal substance misuse behaviour is changing, with the emergence of synthetic drugs such as mephedrone, also referred to as 'Meow Meow' or 'M-Cat'. A Home Office report on the use of khat, a shrub which is chewed for its stimulatory effects predominately by people from east Africa and the Arabian peninsula, found that in four UK cities (London, Bristol, Birmingham, and Sheffield) 34% of the 602 Somalis interviewed reported using the plant leaves (<http://webarchive.nationalarchives.http://rds.homeoffice.gov>).

Opioid use among pregnant women is increasing (Epstein et al., 2013, Desai et al., 2014, Bateman et al., 2014, Salihu et al., 2015). National trend data from United States showed that from 1998 to 2009, opioid use was documented in 138,224 of 55,781,966 pregnancy-related inpatient hospitalizations (25 cases per 10,000 discharges). A statistically significant downward trend occurred from 1998 to 2001, whereas from 2002 to 2009 there was a statistically significant upward trend. The increasing trend in opioid use from 2002 to 2009 is notably higher for whites compared with blacks and Hispanics (Salihu et al., 2015). The 2009 National Survey on Drug Use and Health, the primary source of statistics on illicit drug use in the United States, estimates that 4.5% of pregnant women (15–44 years) used illicit drugs in the month before the survey (National Survey on Drug Use and Health: 2010. www.oas.samhsa.gov). One recent study found that prevalence of chronic medical use of prescription narcotics during pregnancy increased significantly from approximately 2.5 per 1,000 deliveries in 2000 to over 10.0 per 1,000 deliveries in 2008. (Kellogg et al., 2011) This is consistent with the documented increase in therapeutic and nonmedical use of prescription pain relievers in the United States.(Okie, 2010, Manchikanti et al., 2010). Data from Washington state showed that drug exposure and neonatal abstinence syndrome rates increased significantly between 2000 and 2008, neonatal abstinence syndrome rates were

higher than national figures (3.3 compared with 2.8 per 1,000 births in 2008; $P<0.05$). The proportion of neonatal abstinence syndrome-diagnosed neonates exposed prenatally to opioids increased from 26.4% in 2000 to 41.7% in 2008 ($P<0.05$). Compared with unexposed neonates, drug-exposed and neonatal abstinence syndrome-diagnosed neonates had a lower mean birth weight, longer birth hospitalization, were more likely to be born preterm, experience feeding problems, and have respiratory conditions (Creanga et al., 2012). A recent large national cohort of nearly 300 centres in the United States, found increases from 2004 through to 2013 in patient admissions, length of stay and resource utilization for infants admitted to NICUs with the neonatal abstinence syndrome (Tolia et al., 2015)

1.3.2 SIDS and substance misuse during pregnancy

1.3.2.1 Cocaine

Some reports suggested that the SIDS rate was higher in infants who were exposed to cocaine prenatally. In one report, the SIDS rate was 15% amongst 66 infants who were prenatally exposed to cocaine compared to 4% amongst infants exposed to opiates (Chasnoff et al., 1989). A study in California found that of the 1137 infants born of substance-abusing women (16% of total births), 85% were exposed to cocaine. Ten died of SIDS at an incidence rate of 8.8/1000 (Durand et al., 1990) which was significantly higher than the incidence of SIDS among the 5946 infants who had no drug exposure (1.3/1000) (Durand et al., 1990). In contrast, in a study of 175 cocaine-abusing mothers and 821 controls, only one infant of the cocaine abusing mothers died, yielding a SIDS incidence of 5.6/1000, which was similar to the incidence of 4.9/1000 amongst non-exposed offspring (Bauchner et al., 1988). In addition, in a study of 1780 SIDS cases from a population of more than a million infants in New York City between 1979 and 1989, SIDS rates were 5.89 per 1000 births for drug-

exposed infants compared to 1.39 per 1000 control infants (Kandall et al., 1993). When adjustment was made for confounders, the association between increased SIDS rates and drug use remained significant for opioid use (methadone, heroin, or methadone and heroin), but not for cocaine alone or cocaine with opioids. A subsequent meta-analysis of 10 studies reported that the odd ratio (OR) of SIDS in infants born to mothers who used cocaine during pregnancy was 4.10 (95% confidence interval [CI]: 3.17–5.30) (Fares et al., 1997), but there was no increased risk of SIDS related to prenatal cocaine exposure compared to exposure to other drugs. In addition, it was emphasized that as cocaine use is correlated with many potential risk factors, large sample sizes and multivariate statistical techniques are needed to determine whether cocaine use is an independent risk factor for adverse neonatal outcomes. In one series, (Frank et al., 1988) cocaine users were significantly less likely than nonusers to be married, but were more likely to have sexually transmitted diseases, prior low birth weight infants, spontaneous and elective abortions, and greater use of alcohol, cigarettes, and other illicit drugs during pregnancy.

1.3.2.2 Opiates

Infants born to opiate-abusing mothers have been shown to be five to ten times more likely to be at risk of SIDS than the general population (Ward et al., 1990). A study from New York examining a cohort of 383 infants born of substance-abusing mothers recorded a SIDS incidence of 20.9 per 1000 infants (Rajegowda et al., 1978). The study found that no SIDS deaths were attributable to heroin use alone, but 4 of 106 infants who were born to mothers who abused heroin and methadone died of SIDS (Rajegowda et al., 1978). The other four cases of SIDS were born to 182 mothers who were on a methadone treatment program (Rajegowda et al., 1978). Chavez et al reported 17 cases of SIDS in infants born to 688 drug-using mothers (24.7 per 1000 births) (Chavez et al., 1979). Although 14 of the 17 mothers of

SIDS victims were enrolled in methadone treatment programs, the majority of the mothers continued to use other drugs such as heroin, cocaine, diazepam, and amphetamines. A retrospective study of all live births to women in New South Wales during the period encompassing 1995–2002 found that the infant mortality rate was high amongst infants whose mothers were on methadone during pregnancy (24.3 per 1000 live born infants) (Burns et al., 2010)

1.3.2.3 Marijuana (cannabis)

Marijuana is the most frequently used illicit substance in the Western world; 32% of those living in urban areas in the United States (US) were reported to use marijuana (Streissguth et al., 1991). Maternal use of marijuana, however, does not appear to increase the risk of SIDS. In a case-control study in southern California examining 239 infants who died of SIDS and 239 matched healthy infants, no association between maternal recreational drug use and SIDS was found (Klonoff-Cohen and Lam-Kruglick, 2001). In the same study, It was noted that paternal marijuana use during conception (OR = 2.2, 95% CI: 1.2–4.2), pregnancy (OR = 2.0, 95% CI: 1.0–4.1), and postnatally (OR = 2.8, 95% CI: 1.1–7.3) was significantly associated with an increased risk of SIDS after adjusting for paternal smoking and alcohol abuse. The results of that study, however, were from telephone interviews of parents 6 to 12 months after the infant's death, and it would be important to undertake further investigation to confirm or refute this association.

In a study carried out in the UK (the Avon Longitudinal Study of Pregnancy and Childhood), the use of cannabis during pregnancy was not associated with an increased risk of perinatal mortality or morbidity; however, frequent and regular use of cannabis during pregnancy was associated with a small but statistically significant reduction in birthweight (Fergusson et al.,

2002). The adjusted mean birth weights of babies who are born to women who used cannabis at least once per week before and throughout pregnancy were 90 g lighter than offspring of other women (Fergusson et al., 2002). In a study in South London, (Sherwood et al., 1999) cannabis use in early pregnancy was also associated with a reduction in birth weight, a lower gestational age at delivery, and an increased risk of prematurity.

1.3.2.4 Stimulants

Twenty million people consume khat on a daily basis in Africa, and a further 10 million people worldwide may use it regularly (Matloob et al., 2010). The effect of chewing khat leaves produces a stimulatory and euphoric sensation similar to that produced by a mild amphetamine. Although, there are no studies reported which have explored the association between maternal use of Khat during pregnancy and subsequent SIDS, use of khat is associated with low birth weight, (Abdul Ghani et al., 1987) a risk factor for SIDS.

Mephedrone (4-methylmethcathinone) is a novel synthetic stimulant drug that has recently become popular in the UK and Europe. A recent survey found that mephedrone was the sixth most frequently used drug after tobacco, alcohol, cannabis, cocaine, and 3,4-methylenedioxymethamphetamine. (Winstock et al., 2011) The survey highlighted that the majority of ephedrine abusers were of young age; the drug has comparable abuse potential to cocaine. (Winstock et al., 2011). There are no reports investigating ephedrine use and SIDS.

1.3.3 Respiratory control abnormalities in infants of substance-abusing mothers

A variety of respiratory abnormalities have been reported in infants of substance-abusing mothers. A significantly smaller fall in end-tidal carbon dioxide levels in response to a hypoxic challenge, and a significant impairment in arousal responses to hypoxia in infants of substance-abusing mothers have been reported (Ward et al., 1992). Reduced carbon dioxide sensitivity in newborns of substance-abusing mothers has also been highlighted (Wingkun et al., 1995). It has been postulated that ventilatory control abnormalities could lead to abnormal sleeping ventilatory patterns, (Ward et al., 1986) which might contribute to the increased risk of SIDS among infants of substance-abusing mothers. Indeed, in one study, (Ward et al., 1986) 32% of pneumograms from infants of substance-abusing mothers were abnormal compared to 9.3% of pneumograms among controls. The infants of substance-abusing mothers had longer sleep times, a higher total duration of apneas greater than or equal to 6 seconds, and more periodic breathing (Ward et al., 1986).

Abnormalities specific to certain types of prenatal drug exposure (including cocaine and methadone) have been reported. Infants prenatally exposed to cocaine had a higher incidence of cardiorespiratory pattern abnormalities; that is, more frequent apneas and greater intervals of periodic breathing than infants exposed antenatally to methadone (Chasnoff et al., 1989). Apnea density and episodes of periodic breathing exceeded the 95th percentile for normal infants among 38% of cocaine-exposed infants, but only 6% (one in 18) of methadone-exposed infants (Chasnoff et al., 1989). In addition, five infants exposed to cocaine (but no infants exposed to methadone) had apnea (Chasnoff et al., 1989). A higher frequency of respiratory pauses and a greater decrease in minute ventilation in response to facial air stream stimulation has been reported in infants exposed prenatally to cocaine compared to controls (Chen et al., 1991).

Infants exposed to methadone in-utero were demonstrated to have decreased sensitivity to a carbon dioxide challenge when compared to controls, as measured by the slope of the ventilatory response curve during the first weeks after birth (Olsen and Lees, 1980). The depressed ventilatory response to carbon dioxide lasted on average for 15 days after birth (Olsen and Lees, 1980).

There are however no longitudinal data on the response of SM infants to hypercarbia nor data on their response to hypoxia. Hence, a further aim of this thesis is to study this.

The majority of women who engage in substance misuse also smoke, and thus some of the respiratory control abnormalities seen in infants of substance-misusing women may, at least in part, relate to their smoking. Therefore, it would be important to compare the responses of infants of mothers who substance misused to those who only smoked

1.4 Peripheral chemoreceptors and ventilatory response to hypoxia in newborns and infants

Peripheral chemoreceptors monitor changes in arterial blood O_2 , and within seconds after the onset of hypoxia they trigger cardiorespiratory changes (i.e., increase in breathing and blood pressure), which are important for maintaining O_2 homeostasis. Conventionally, peripheral chemoreceptors are thought to comprise carotid and aortic bodies. However, tissues similar to carotid and aortic bodies have also been described in thorax and abdomen, which are often called “paraganglion” and may serve as additional chemoreceptors (Easton and Howe, 1983) (Deane et al., 1975)

Newborns and infants have a biphasic response to hypoxia. The biphasic response to hypoxia consists of an immediate (30 seconds-1 min) increase followed by later decline (5 min) in minute volume to often below normoxic levels (Neubauer et al., 1990). It was first described in the newborn by Cross et al in 1952 (Cross and Oppe, 1952). In their study, Cross and Oppe

showed that infants breathing 15% oxygen had an immediate but a rather transient increase followed by decline in minute ventilation. They suggested that despite the evidence of active chemoreceptor response to hypoxia, it was not effective in maintaining hyperventilation if the hypoxia was prolonged beyond two minutes (Cross and Oppe, 1952). The authors suggested that the late depression in ventilation was secondary to anoxic effects on the medullary centres. Similar findings of biphasic ventilator response to hypoxia were also described in other studies in the newborn by the same group (Cross et al., 1954, Cross et al., 1958) and by other in term (Brady and Ceruti, 1966) and preterm infants (Rigatto et al., 1975, Nock et al., 2004, Martin et al., 1998). The biphasic response to hypoxia was also described in a series of animal studies (Rigatto and Brady, 1972a, Gershan et al., 1994, Haddad et al., 1982, Mortola et al., 1989, Rigatto et al., 1988).

Adult's hypoxic ventilatory response differs from the biphasic response described above in newborns and infants both in terms of the magnitude of change in minute volume and the time course of the response (Easton et al., 1986, Weil and Zwillich, 1976). The ventilatory response to hypoxia in adults consists of an initial increase in ventilation that peaks within 3–5 min and is sustained for approximately 15–30 min before a subsequent decline to pre-hypoxic baseline values (Douglas et al., 1982).

The initial increase in ventilation in response to hypoxia newborns and infants is mediated through the peripheral arterial chemoreceptors located in the carotid and aortic bodies. Hypoxia is initially associated with an increase in both respiratory frequency and tidal volume (Rigatto et al., 1975, Rigatto, 1977).

Different mechanisms have been suggested to explain the cause of the ventilatory depression that follows the initial increase in ventilation. Cross et al (Cross et al., 1954), first suggested that the late decline in ventilation was likely due to the central depressant effects of hypoxia.

He later suggested that a decline in metabolism was also a possible cause (Cross et al., 1958). Subsequent studies have attributed the late decline in ventilation during hypoxia to a variety of mechanisms including, a decrease in metabolism (Mortola and Matsuoka, 1993, Mortola et al., 1989), change in breathing pattern (Rigatto and Brady, 1972b), decrease in compliance (LaFramboise et al., 1983) or a decrease in temperature (Adamsons, 1959, Cross et al., 1959). LaFramboise et al (LaFramboise et al., 1983) suggested the influence of mechanical factors since their study in a monkey preparation showed undiminished central output in the presence of decreased ventilation during late hypoxia. Tidal volume decreased significantly and was the primary factor responsible for the decrease in ventilation, with frequency remaining elevated. Rigatto et (Rigatto et al., 1988) found similar results in the unanesthetized kitten, with a predominant decrease in V_T . Similarly, a decrease in compliance due to bronchoconstriction was suggested, but the observations of a bronchodilatory effect in response to hypoxia in 70 of 76 airways studied with high-resolution tomography seem to make this possibility unlikely (Wetzel et al., 1992). A decrease in temperature may accentuate the biphasic response, with a more pronounced late decrease in ventilation, but some studies have demonstrated that the response clearly occurs during thermo neutrality (Brady and Ceruti, 1966). Many studies have demonstrated a decrease in metabolism during hypoxia, in both animals (Gershan et al., 1994, Haddad et al., 1982, Mortola et al., 1989) and humans (Cross et al., 1958). A decrease in metabolism would induce a corresponding decrease in ventilation mediated through a decrease in CO_2 production (Mortola and Matsuoka, 1993). Therefore, the more likely explanation for the late decrease in ventilation during hypoxia appears to be a decrease in metabolism, a central respiratory depression, or both.

The central sites responsible for the hypoxic ventilatory decline have been suggested to exist in the upper medulla by Cross et al (Cross et al., 1954) or above the midcolliculi by Dawes

and co-workers (Dawes et al., 1983) . In the experiments of Dawes et al, (Dawes et al., 1983) hypoxia stimulated breathing in the midcollicularly transected fetal sheep, suggesting the possibility of an inhibitory action of hypoxia on a center located rostral to the section level. Johnston and co-workers (Gluckman and Johnston, 1987, Johnston and Gluckman, 1993), have localized a specific area in the rostral lateral pons, in the region of the lateral parabrachial and Kolliker-fuse nuclei, which appears to be involved in the central inhibition of breathing during hypoxia in the fetal lamb. After electrolytic lesions of this area, hypoxemia stimulated rather than abolished breathing in the fetus. This appears likely to be the site of action for neurotransmitters that are released by hypoxia and inhibit breathing.

There is clear evolution of the hypoxic ventilatory response from fetal life to neonatal period/infancy and later on in adults: hypoxia causes ventilatory depression in the fetus(Alvarez et al., 1992), stimulates and depresses ventilation it in the newborn (Cross and Oppe, 1952, Cross et al., 1954, Rigatto et al., 1975, Rigatto, 1977, Rigatto and Brady, 1972a), also stimulates and depresses ventilation it in the adult, but less so than the newborn (Easton et al., 1986, Weil and Zwillich, 1976). The evolution is therefore one of gradual diminishing degree of ventilatory depression with increasing age.

There is conflicting evidence regarding the time of maturation of the ventilatory response to hypoxia in infants and the actual age at which infant's hypoxic ventilatory response becomes similar to the adult response. Some studies have shown that the immature biphasic HVR existed up to 2 months of age (Cohen et al., 1997, Martin et al., 1998). In another study, the immature biphasic response to hypoxia persisted until 5-6 months of age (Richardson et al., 2007)

It is therefore important in assessing the response to hypoxia in SM and S infants to recruit a control group and this will be done in this thesis.

1.5 Central chemoreceptors and the responses to hypercapnia in newborns and infants

The sensitivity of the central chemoreflex may be estimated using either rebreathing or steady-state methods to increase carbon dioxide while maintaining hyperoxia to reduce the peripheral-chemoreflex ventilatory response (Cunningham, 1987). While some investigators report no difference in central-chemoreflex sensitivities between the two methods, (Read, 1967, Clark, 1968, Oren et al., 1991, Soto Campos et al., 1996), others report higher sensitivities for rebreathing compared to steady-state methods (Berkenbosch et al., 1989, Bourke and Warley, 1989)

The ventilatory response to hypercapnia is dependent on the relative size and maturity of the species at birth, and generally increases with postnatal age (Bonora et al., 1994), in part as a result of increasing sensitivity of both central and peripheral chemoreceptors (Davis et al., 2006) (Hanson et al., 1989). Newborn animals and premature and mature infants increase ventilation when breathing low concentrations of CO₂ (Guthrie et al., 1980) (Haddad et al., 1980, Rigatto et al., 1980) (Rigatto et al., 1981). Tidal volume is increased with little or no effect on breath timing, so that the calculated "central inspiratory drive" (V_T/T_I) is increased. The hypercarbic ventilatory response of most newborn species is reduced compared with that of adults (Carroll et al., 1993, Carroll and Fitzgerald, 1993, Davis et al., 2006), unlike hypoxia, it is sustained over time by a persistent increase in tidal volume (V_T), despite a gradual decline in the initial respiratory frequency (f) (Bonora et al., 1994) (Cummings and Frappell, 2009).

Inhalation of very low concentrations of CO₂ (0.3-1.2%) in infants was shown to make periodic and irregular breathing more regular; breath frequency increased and tidal volume fell (Rigatto et al., 1980). When breathing was regular, CO₂ inhalation tended to increase

tidal volume with little change of breath timing (Kalapesi et al., 1981). This shows that there is significant interaction between the central effects of CO₂ and the neural mechanisms that determine the ongoing respiratory pattern.

There is conflicting evidence regarding the influence of sleep stage on the ventilatory response to hypercapnia. A difference in resting ventilation and in the response to CO₂ when REM (Rapid Eye Movement) and NREM (Non-Rapid Eye Movement) sleep episodes were compared was not apparent in newborn monkeys before 3 weeks of age (Guthrie et al., 1980), nor in premature infants (Kalapesi et al., 1981, Rigatto et al., 1980) or term infants until about 3 months (Haddad et al., 1980). A small increase of ventilation during sleep was noted in 11-week-old infants in one study (Fagenholz et al., 1976). The definitive sleep pattern is laid down gradually over several months, and there must be some difficulty in distinguishing each sleep state clearly in the very young infant. Oxygen consumption and the thermoregulatory response to cooling were also similar in both sleep states in premature infants (Darnall and Ariagno, 1982); this differs from the adult, where O₂ consumption is higher and the fall of body temperature greater during cooling in REM sleep. Thus, sleep-related mechanisms that modulate breathing, metabolism, thermoregulation, and the response to CO₂ do not operate fully in the newborn primate (Walker, 1984).

Infants who had had an apnea requiring resuscitation had a greater ventilatory response to inhaling 2% CO₂ than age-matched controls (Haddad et al., 1981). These infants also had higher resting heart rates and shortened QT intervals; it was suggested that increased sympathoadrenal activity caused the raised CO₂ response, since infusion of catecholamines in the adult is known to increase the slope of the CO₂ response curve. Others have not observed altered CO₂ sensitivity when infants showing prolonged (>20 sees) spontaneous apnea were compared to those with regular breathing (Fagenholz et al., 1976). Infants with persistent hypoventilation during sleep had a very low sensitivity to CO₂, an effect attributed to

impairment of central CO₂ sensitivity, because peripheral chemoreceptor responses appeared to be normal (Lagercrantz et al., 1980, Shannon et al., 1976) (Wells et al., 1980)

Data on ventilatory responses to hypercarbia are sparse at the peak age of SIDS thus it would be important to assess the infants response to hypercarbia in the perinatal period and at the peak age for SIDS.

1.6 Summary

Infants of substance abusing mothers have a number of risk factors for Sudden Infant Death Syndrome (SIDS), but after controlling for other high risk variables, their rate of SIDS is higher than that of the general population. There is some evidence to suggest that infants of substance abusing mothers have a disruption in the maturation of respiratory control. The profile of drug abuse, however, has changed since those studies and there are no data from infants of substance abusing mothers at the high-risk age for SIDS.

Women who substance-misuse during pregnancy, almost always smoke and infants of mothers who smoke during pregnancy are also at high risk for SIDS. Thus, essential to understanding why infants of substance abusing mothers are at increased risk of SIDS, is to compare their results with infants whose mothers neither substance abused nor smoked (controls), but also to those of infants whose mothers smoked during pregnancy. There are no data available comparing chemoreceptor responses in new-borns and infants of smoking, substance-abusing and non-smoking/non-substance-abusing (controls) mothers. There is also limited longitudinal data on chemoreceptor responses in infants of smoking and substance-abusing mothers. By assessing the infants twice, I will determine if there are changes with increased postnatal age and whether infants at high risk can be detected prior to maternity unit discharge.

1.7 Hypotheses

- Infants of substance abusing mothers and infants of mothers who smoke will have poorer ventilatory responsiveness to hypercarbia and hypoxia and reduced chemoreceptor sensitivity compared to controls
- Impairment of ventilatory responsiveness and reduced chemoreceptor sensitivity will be greater in the infants of mothers who both substance abuse and smoke compared to those whose mothers only smoke

1.8 Aims

To assess the correlation between maternal reported smoking and urinary cotinine levels

To describe the profile of maternal substance-misuse

To study the effect of maternal smoking and substance misuse during pregnancy on the ventilatory response to hypercapnia in the perinatal period

To study the effects of maternal smoking and substance misuse during pregnancy on infant's ventilatory response to hypercapnia at the peak age of SIDS

To study the effects of maternal smoking and substance misuse during pregnancy on infant's ventilatory response to hypoxia in the perinatal period

To study the effects of maternal smoking and substance misuse during pregnancy on infant's ventilatory response to hypoxia at the peak age of SIDS

To study the maturation of the ventilatory response to hypercapnia with increasing postnatal age

To study the maturation of the hypoxic ventilatory response with increasing postnatal age

Chapter 2

Methods

2 Methods

2.1 Subjects

2.1.1 Inclusion criteria

Infants were eligible for entry into the study if they were born at 36 weeks of gestational age or greater at King's College Hospital NHS Foundation Trust. Informed written parental consent was obtained and the study was approved by the Guy's and St Thomas's Hospitals NHS Foundation Trust Research Ethics Committee.

Three groups of mothers/infants were recruited in the antenatal and/or immediate neonatal period:

1. Mothers with a history of substance misuse during pregnancy were approached antenatally in a dedicated substance misuse clinic (SM infants)
2. Infants whose mothers gave a history of smoking during pregnancy. Smoking was defined as any history of daily smoking regardless of the number of cigarettes smoked/day (S infants)
3. Infants whose mothers neither smoked nor misused substances during pregnancy (controls)

2.1.2 Exclusion criteria

Infants were not included in the study if they had congenital anomalies, significant cardiovascular or neurological abnormalities and a respiratory illness.

2.2 Protocol

Infants were first studied in the immediate newborn period before discharge from the maternity unit. The infants were also studied at 6-12 weeks of age in a purpose built infant assessment unit.

2.3 Assessment of exposure to smoking and the type and amount of substance misuse

2.3.1 Smoking

Mothers with history of smoking were asked about the average number of cigarettes smoked per day. Maternal self-reported number of cigarettes smoked was related to the results of cotinine analysis.

2.3.1.1 Laboratory measurement of cotinine

The detection of smoking by measurement of nicotine metabolites such as cotinine is favoured over other methods, such as carboxyhaemoglobin or thiocyanate determinations. This is partly because cotinine and other nicotine metabolites are tobacco specific, whereas carboxyhaemoglobin and thiocyanate may be present as a result of exposure to other environmental factors. Moreover, cotinine has a longer half-life of (2-3 days) than nicotine or carboxyhaemoglobin. The measurement of cotinine is an aid to confirm the smoking status as well as quantifying the magnitude of smoking.

Maternal and infant urine cotinine levels were measured in the immediate perinatal period

Urine sample were collected into a universal container without preservative. Samples were assayed immediately or aliquoted and stored at -20° C. Samples are centrifuged before analysis. Cotinine reagent was supplied by Siemens Healthcare Diagnostics Ltd. This analyte was measured on the Siemens Immulite 2000 analyser. The assay is a solid phase competitive

chemiluminescence immunoassay for the measurement of cotinine in human serum or urine. It utilises a rabbit polyclonal antibody, which is bound to a bead and the cotinine is conjugated to alkaline phosphatase in the form of a reagent. These reagents were placed on a Siemens Immulite 2000. The sample and reagent are incubated together with the coated bead for 30 minutes. During this time, cotinine in the sample and cotinine conjugated to alkaline phosphatase in the reagent compete for binding sites on the beads. Unbound patient sample and excess reagent were then removed by centrifugal washes. Finally, chemiluminescent substrate was added to the reaction tube containing the bead and the signal was generated which was in proportion to the bound enzyme and inversely proportional to the amount of cotinine present in the sample. The cut-off limit for detection of cotinine was 10ng/ml.

2.3.2 Substance misuse

The substance-misuse profile of mothers in the substance misuse group was reviewed. Details of the maternal methadone dose during pregnancy were obtained from the medical records of mothers on “prescribed” methadone. Results of the urine analyses performed during pregnancy were reviewed.

2.3.2.1 Urine drug screen

In the immediate postpartum period, urine samples were taken from the mothers and newborns for drug screen. Urine samples were collected into universal containers without preservative. No specific patient preparation was required before collecting the samples and no specific timing of sample collection was required. Attempts were made to collect maternal and infants sample close to each other, but this was not always feasible.

Screening of the urines was carried out for cannabinoids, opiates, amphetamines, methadone, cocaine and benzodiazepines. The urine drug assay works on the (CEDIA) Principal Cloned Enzyme Donor Immunoassay. CEDIA technology is based on Beta -galactosidase that has been synthesised in two inactive fragments that spontaneously re-associate to form the active enzyme. The cut-off concentrations used to determine positivity were as recommended by the manufacturer (cocaine 300ng/ml, methadone 100ng/ml, cannabis 25ng/ml, benzodiazepines 300ng/ml and amphetamines 500ng/ml)

2.4 Physiological measurements

Infants were studied in the supine position with their head in the midline. All physiological measurements were carried out when infants were in quiet sleep. Sleep state was determined by observation of the behavioural state (Prechtl et al., 1979). The infant was determined to be in quiet sleep when the eyes were closed, there was no body or eye movement, no vocalization and the respiratory rate was regular.

2.4.1 Hypercarbic challenge

The hypercapnic challenge was delivered via a facemask and pneumotachograph through a custom-made open circuit system with individually adjustable flows of CO₂ and air (Figure 2.1). A bias flow of air was passed through the open circuit, eliminating any dead space. Respiratory flow was measured using an appropriately sized pneumotachograph (Mercury F10L, G M Instruments, Kilwinning, Scotland) with a dead space 0.8ml and a resistance of 0.86 mmH₂O/L/min. A facemask was placed over the infant's mouth and nose. The pneumotachograph was attached to the distal end of the facemask and its distal was connected to a two-way non-rebreathing valve. The pneumotachograph was attached to a differential pressure transducer/amplifier (13-4615-70, Gould, Cleveland OH, USA) and the flow signal was subsequently acquired and displayed in real time on a PC computer running

Spectra software (Grove Medical, London, UK) with 100 Hz analogue to digital sampling (PCI-MIO-16XE-50, National Instruments, Austin TX, USA). Respiratory rate, inspiratory time (T_i) and the time to peak tidal expiratory flow were determined from the flow signal. Tidal volume was determined by digital integration of the flow signal by the acquisition software. A constant flow of medical air from a cylinder was delivered to the inspiratory port of a two-way non-rebreathing valve via a length of wide bore (20mm), low resistance tubing. The inspired air could be enriched with a variable concentration of carbon dioxide (CO_2) from a cylinder, the flow being controlled by a rotameter. Inspired and expired gases were sampled continuously using a small cannula inserted through the wall of the facemask and positioned close to the infant's mouth and measured using a capnography (CO_2 SMO capnography (Respironics UK, Chichester, UK). The capnograph was calibrated with certified calibration gas (5% CO_2 /95% air, BOC Gases, UK) prior to each measurement. Inspired CO_2 and end-tidal CO_2 (ETCO_2) levels were derived from the continuous capnogram recording, which was recorded simultaneously with respiratory flow and displayed in real time throughout the study. Assessments were made at three levels of inspired CO_2 (0% (baseline), 2% and 4%). The sequence for administering the level of inspired CO_2 was randomised between each infant. Those levels were chosen to allow measurement of changes in respiration elicited by linear increases in the inspired CO_2 , without generating significant behavioural arousal and are in line with previous studies (Cohen and Henderson-Smart, 1994, Frantz et al., 1976). Each mixture of CO_2 was titrated and a stable inspiratory CO_2 concentration was achieved within the delivery tubing, as assessed by the capnograph readout, before the infant was connected to the breathing circuit. The infant breathed the air/ CO_2 mixture for at least five minutes to allow ventilation and ETCO_2 to reach steady state as assessed from the real-time display using the Spectra software (Grove Medical, London,

UK). There was a five minutes washout period between each level of inspired CO₂ to ensure that the infant's breathing returned to baseline

The following outcomes were assessed with the hypercarbic challenge:

1. Baseline respiratory rate, tidal volume, minute volume and time to peak tidal expiratory flow
2. The percent change in minute ventilation related to the percent of inspired carbon dioxide
3. The slope of the ventilatory response to hypercarbia, this is the gradient of the line of best fit of minute volume against the inspired CO₂ level (Figure 2.3).
4. The mean inspiratory flow (MIF) change was reported as the percentage increase from the baseline MIF to the MIF at 4% CO₂.
5. The time constant of the response to hypercarbia, defined as the time taken to achieve 63% of the maximal response to 4% CO₂ (Figure 2.4)

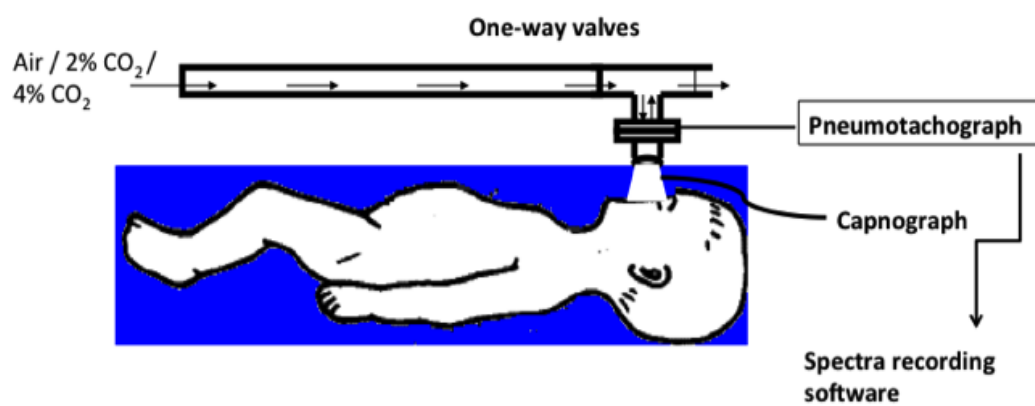


Figure 2.1 Infant position and the equipment used in the hypercarbic challenge

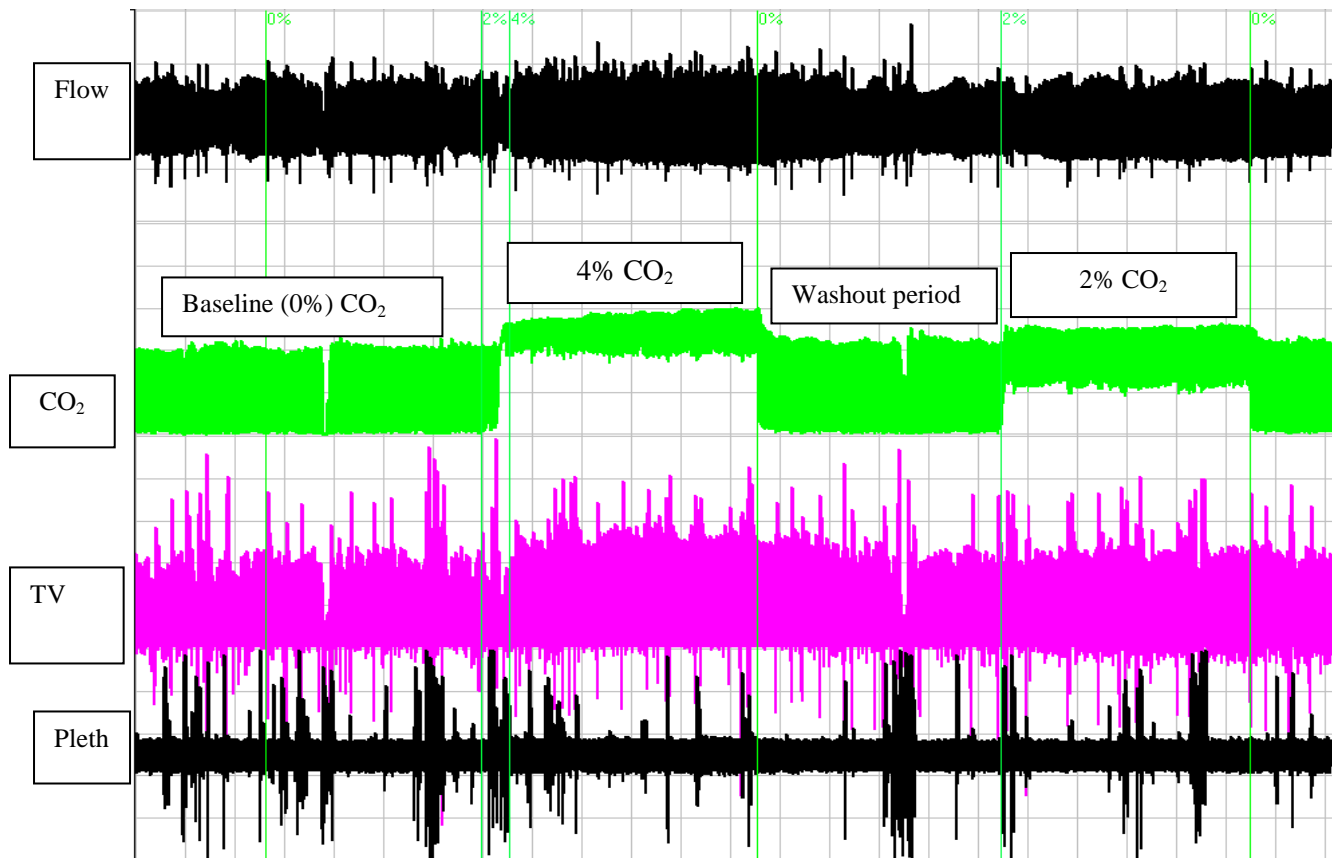
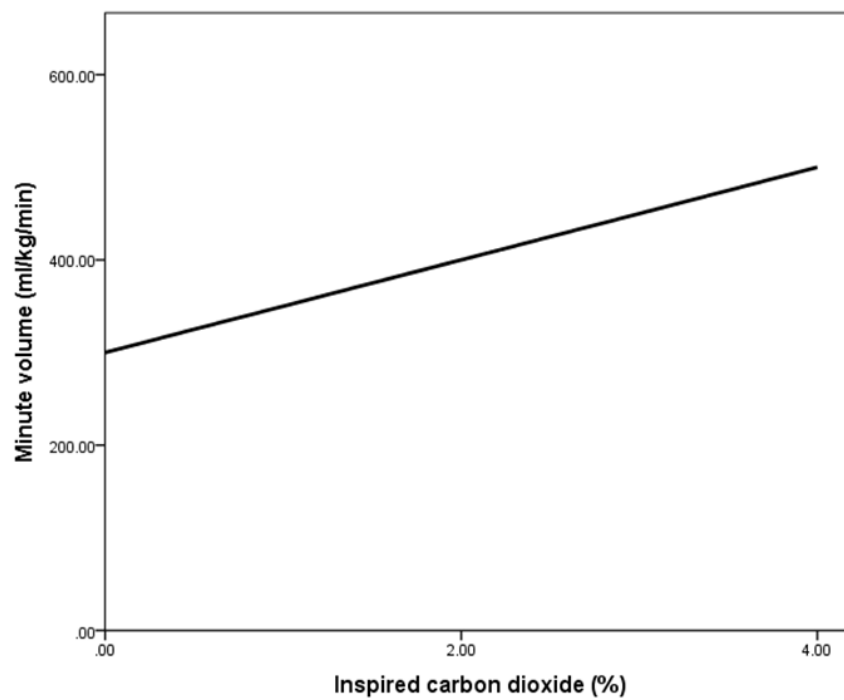


Figure 2.2 Spectra recording during hypercarbic challenge

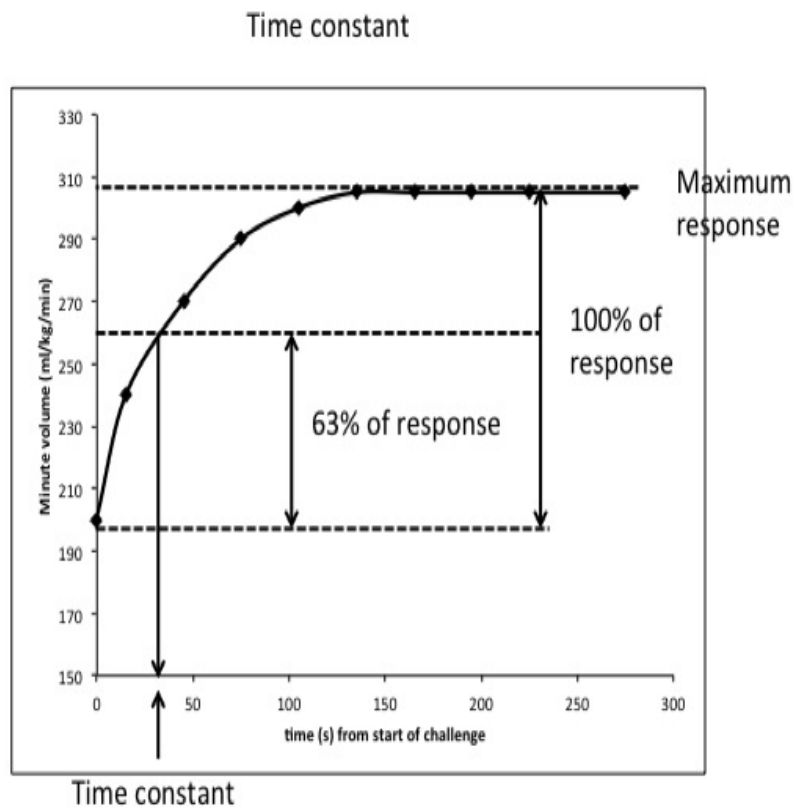
The diagram illustrates the recording on spectra acquisition software during the hypercarbic challenge. The flow signal (top-black colour) was derived from the pneumotachograph. Tidal volume (Purple colour) was derived from digital integration of the flow signal and later adjusted for body weight. Inspired CO₂ (Green colour) was derived from the capnography using a CO₂ sampling line inserted through the facemask. During the study displayed above, the infant was switched from breathing 0% CO₂ TO 4% CO₂ for a period of 5 min. A five washout period was shown before the level of inspired CO₂ was changed to 2%.The plethysmography wave shows represents the output from the Masimo-set pulse Oximetry

Figure 2.3 The slope of ventilatory response to hypercarbia



The slope of ventilatory response to hypercarbia determined by the gradient of the line of best fit of minute volume against percentage of inspired CO₂

Figure 2.4 Hypercarbic challenge outcomes.



The figure illustrates the measurement of the time constant of the response to the hypercarbia

2.4.2 Hypoxic challenge

The hypoxic challenge was delivered via a facemask and custom-made open circuit system using 15% oxygen in balanced nitrogen from a cylinder (BOC Gases, UK). The facemask was placed over the infant's mouth and nose; therapeutic putty was put around the rim of the facemask to ensure an airtight seal around the face. Respiratory flow was measured using an appropriately sized pneumotachograph connected to the facemask (Mercury F10L, G M Instruments, Kilwinning, Scotland) with a dead space 0.8 ml and a resistance of 0.86 cmH₂O/L/second. The distal end of the pneumotachograph was connected to a two-way non-rebreathing valve contained within the open circuit system. A constant flow of medical air from a cylinder (BOC Gases, UK) was delivered to the inspiratory port of the two-way non-rebreathing valve via length of wide bore (20mm), low resistance tubing. The pneumotachograph was attached to a differential pressure transducer/amplifier (13-4615-70, Gould, Cleveland OH, USA). Data were acquired and displayed in real time on a PC computer running Spectra software (Grove Medical, London, UK) with 100 Hz analog to digital sampling (PCI-MIO-16XE-50, National Instruments, Austin TX, USA). Tidal volume was determined by digital integration of the flow signal by the acquisition software. Inspired and expired gases were sampled continuously using a small cannula inserted through the facemask and positioned close to the infant's mouth. Oxygen saturation and heart rate were measured using a pulse oximeter (Masimo rainbow SET Pulse Oximetry).

During quiet breathing, the infants were switched from breathing room air to 15% oxygen in nitrogen (BOC Gases, UK). The final minute of tidal breathing in air was used as the baseline value. The hypoxic challenge was maintained for five minutes, but terminated if the oxygen saturation level fell below 85%. Infants respond to hypoxia with a biphasic response (Figure 2.6). The responses to the hypoxic challenge were determined by:

- 1) The magnitude of increase in minute ventilation from baseline to the peak ventilation
- 2) The magnitude of decline in minute volume from the peak to the lowest minute volume
- 3) The change in the oxygen saturation level from baseline to the lowest oxygen saturation level.
- 4) Magnitude of increase in heart rate from baseline to peak in response to hypoxic challenge
- 5) Changes in end tidal CO₂ (ETCO₂) with hypoxia
- 6) The rate of decline in minute volume, calculated as the peak minute volume-lowest minute volume divided by the time from peak to the lowest minute volume.

The time to peak minute ventilation was noted, as was the time to the lowest SaO₂.

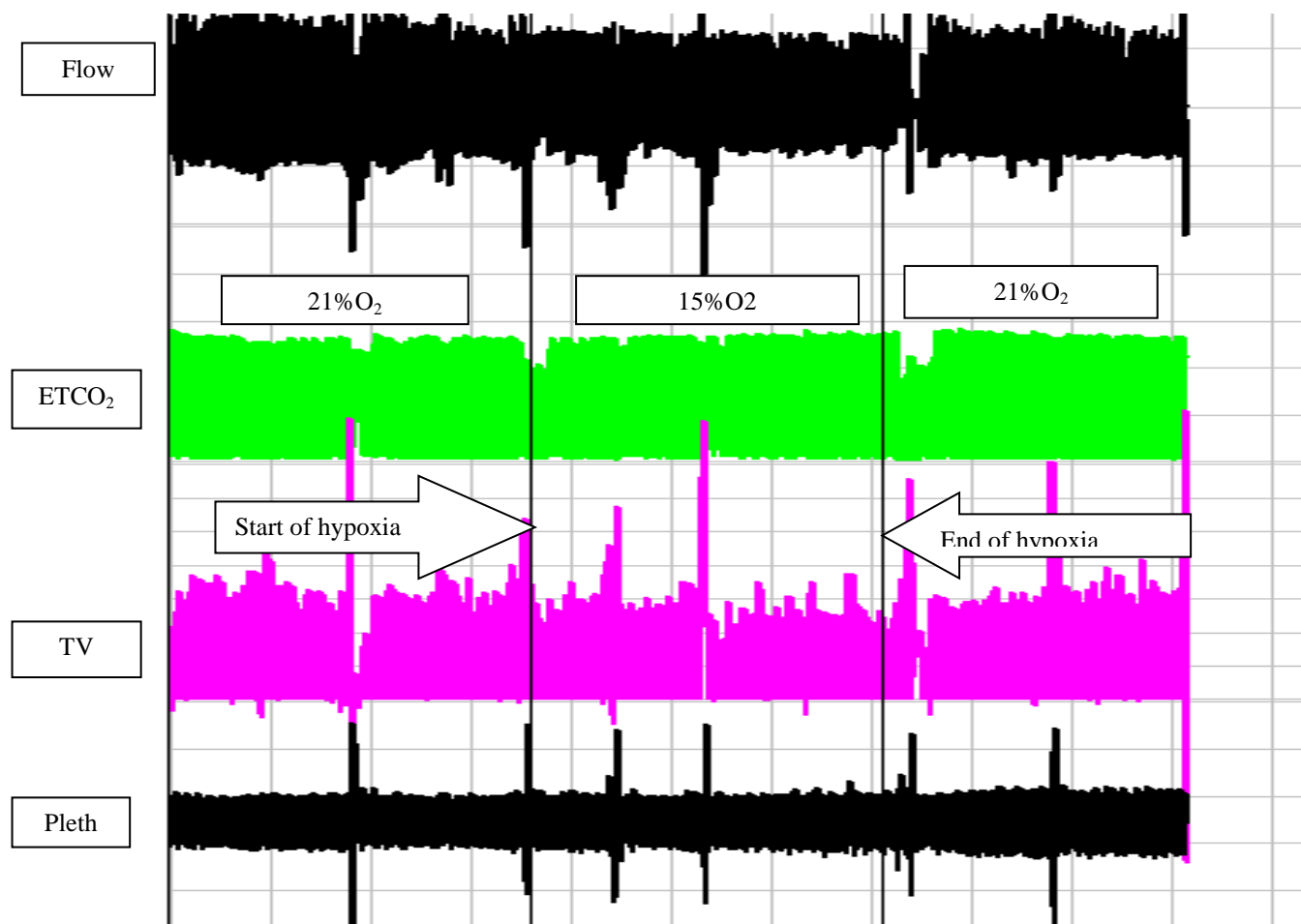
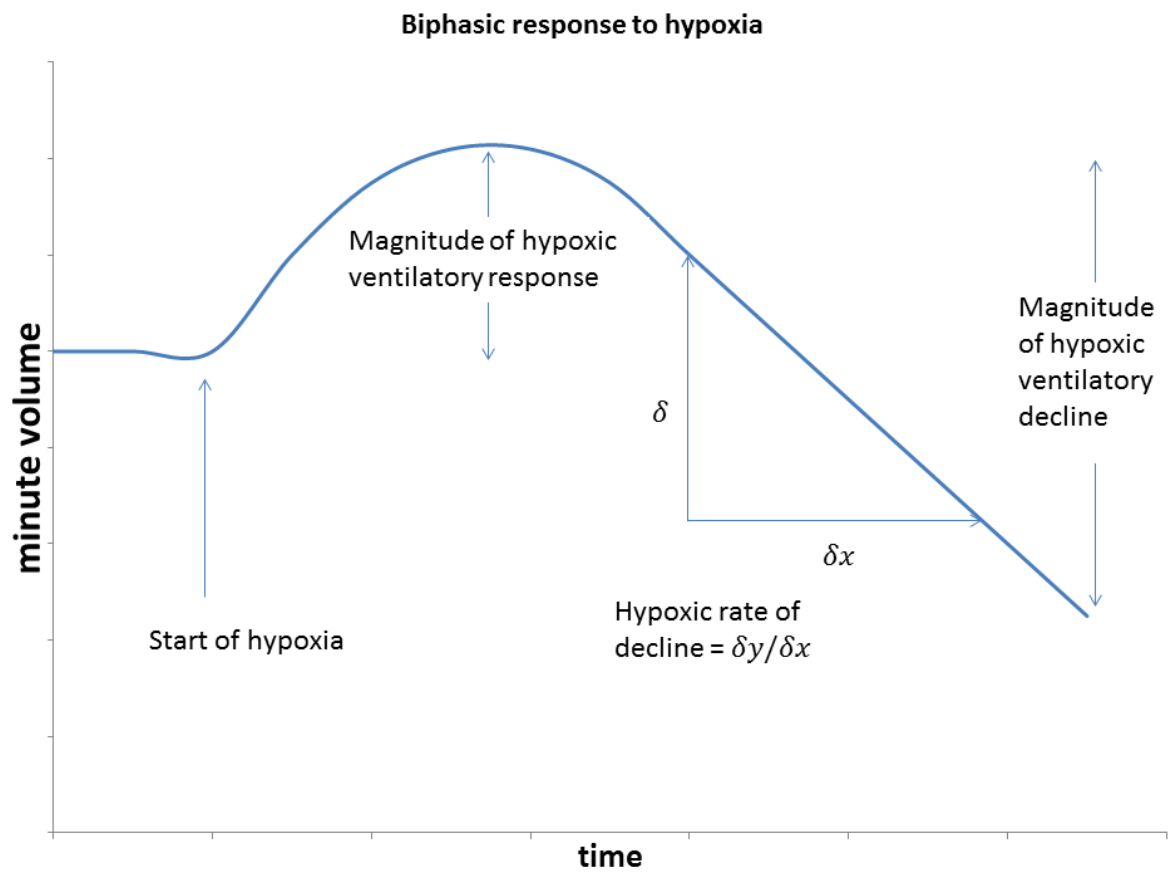


Figure 2.5 Spectra recording during hypoxic challenge

The diagram illustrates the recording on spectra acquisition software during the hypoxic challenge.

The flow signal (top-black colour) was derived from the pneumotachograph. Tidal volume (Purple colour) was derived from digital integration of the flow signal and later adjusted for body weight. ETCO₂ (Green colour) was derived from the capnography using a CO₂ sampling line inserted through the facemask. The infant was switched from breathing room air (arrow) to 15% oxygen for period of five-minutes. The plethysmography wave shows represents the output from the Masimo-set pulse Oximetry

Figure 2.6 Outcomes of the hypoxic challenge



2.4.3 Equipment used for physiological measurements

2.4.3.1 Measurement of flow

Flow was measured using an appropriately sized pneumotachograph (Mercury F10L, G M Instruments, Kilwinning, Scotland) with a dead space 0.8ml and a resistance of 0.86 mmH₂O/L/min inserted between the facemask a two-way non-rebreathing valve. The pneumotachograph was attached to differential pressure transducer/amplifier (13-4615-70, Gould, Cleveland OH, USA)

2.4.3.2 Calibration of the pneumotachograph

Calibration of flow was carried out prior to each measurement. Flow was calibrated using a low flow rotameter (0-12 L/min Platon, Roxspur Measurement & Control Ltd, Bramley, Hants, UK).

2.4.3.3 Linearity of system used to measure flow

To assess pneumotachograph and pressure transducer linearity, air over a range of flows (1 to 10 L/min) was passed through the pneumotachograph and the output plotted against the actual flow delivered by the rotameter. It had a linear response up to 10 L/min for both an inspiratory and expiratory directions of flow.

2.4.3.4 Frequency response of the flow measurement system

Frequency response can be determined by a 'pop test' in which an inflated balloon is fitted over the signal input site and then burst, producing an instantaneous negative step input to the system. The frequency response of the system (f_{3db}) is calculated using the equation $f_{3db} = 1/3 \times T_r$, where T_r (response time) is defined as the time taken for pressure to fall from 90% to 10% of the initial pressure. The frequency response was determined using the pop test method, with the change in pressure against time recorded on a computer (MacBook, Apple Computer Corp, Cupertino, California, USA) using Chart software (Version 5.0, ADInstruments Pty Ltd, Bella Vista, NSW Australia) with analogue-to-digital sampling at 40KHz (Powerlab, ADInstruments Pty Ltd, Bella Vista, NSW, Australia). The frequency response of the flow measurement system (consisting of the pneumotachograph, connecting tubing, pressure transducer for flow, amplifier and computer) was determined by placing one end of the pneumotachograph within an inflated balloon. By partially occluding the other end of the pneumotachograph, a constant background flow was produced, during which the balloon was burst. The response time was 9 milliseconds, giving a calculated frequency response of 37 Hz.

2.4.4 Capnography

Inspired and expired gases were sampled continuously using a small cannula inserted through the wall of the facemask and positioned close to the infant's mouth, and measured using a capnograph (CO₂SMO capnograph; (Respironics UK, Chichester, UK).

2.4.4.1 Calibration of the capnography

The capnography was calibrated with certified calibration gas (5% CO₂/95% air, BOC Gases, UK) prior to each measurement.

2.4.4.2 Linearity of exhaled carbon dioxide measurement

To evaluate the linearity of the capnography various known concentrations of CO₂ were connected to the capnography. The acquired measured values were plotted against known concentration of CO₂; this was observed to be linear for up to 10% CO₂ concentration.

2.4.5 Masimo SET® Pulse Oximetry

This is a noninvasive, arterial oxygen saturation and pulse rate monitor. The monitor features a backlit liquid crystal display (LCD) that continuously displays numeric values for SaO₂ and pulse rate. It provides graphical display of plethysmographic waveform which is displayed continuously on the acquisition software (spectra).

2.4.6 Data acquisition and storage

The data from the pneumotachograph, capnography and the Masimo set monitor were transferred as a digital signal through a RS232 interface. The data was transferred to a PC computer which was preloaded with customised data acquisition software (Spectra, version 3.0.1.4; Grove medical, London, UK). The Spectra software is a type of 'digital chart recorder' and is used to record signals from clinical transducers, patient monitors and life support equipment. In real time the software can capture, display and perform complex analyses and display the results. It can also mark events, replay and re-analyse data. Waveforms were recorded in real time, and displayed in graphical form. The display of recorded data was time-based; hence it was possible to review any period of interest. All datasets were stored with reference to a patient database, which can be reviewed and amended in real time without interruption to the data collection process.

2.5 Sample size

I aimed to assess 20 infants in each of the three groups completing assessments at 6-12 weeks of age as this sample size allowed detection of a difference of one standard deviation in each outcome measurement between the groups with 80% power at the 5% significance level. The magnitude of difference in the ventilatory response to added dead space between newborns of smoking and non-smoking mothers has been previously detected (Bhat et al., 2005). I assumed there would be a 50% drop-out rate between recruitment in the neonatal period and assessment at follow up. Therefore, I planned to recruit 120 infants (40 in each group) for the neonatal studies.

Chapter 3

3 Maternal and infant urinary cotinine and substance misuse screen

3.1 Introduction

The aims of the study were:

- To validate maternal cigarette smoking practices using cotinine levels obtained in the immediate postpartum period
- Compare smoking practices between mothers who substance-misused and smoked those who smoked only and between mothers who smoked tobacco and cannabis and those who smoked tobacco only
- Describe the profile of substance misuse among mothers and infants

3.2 Methods

Antenatal substance-misuse profile of mothers in the SM group was reviewed. Details of the maternal methadone dose during pregnancy were obtained from the medical records for mothers on “prescribed” methadone.. Maternal smoking was defined as daily cigarette smoking regardless of the number smoked/day.

3.2.1 Urine drug screen

In the immediate postpartum period, urine samples were taken from the mothers and infants for drug screen. Urine samples were aliquoted and then stored at -20°C until assayed. Screening of the urines was carried out for cannabinoids, opiates, amphetamines, methadone, cocaine and benzodiazepines as described in chapter 2.

3.2.2 Cotinine analysis

Maternal and infant's urinary samples were tested for cotinine in the perinatal period.

Cotinine reagent was supplied by Siemens Healthcare Diagnostics Ltd. The assay was a solid phase competitive chemiluminescence immunoassay for the measurement of cotinine in the urine as described in chapter 2.

3.2.3 Statistical Analysis

Data were tested for normality using the Kolmogorov-Smirnov test. Non-normally distributed data are presented as median (range). Linear regression analysis was used to assess the correlation between maternal cotinine levels; self-reported number of cigarettes smoked/day and infant's cotinine levels.

Man-Whitney U and Fisher Exact test were used to compare differences between infants of mothers who smoked only (S) and those who smoked and substance misused (SM) and between infants of mothers who smoked tobacco and cannabis and those who tobacco smoked only.

3.3 Results

140 maternal and infant's urinary samples were screened for illicit drugs and cotinine in the perinatal period. Forty-nine mothers tested positive for cotinine with levels above the detection limit of 10ng/ml. Mothers smoked a median of 7 cigarettes/day range (2-20) (Table 3.1). In the control group, twenty pairs of maternal/infant's urinary samples were screened for cotinine with all the samples testing negative (cotinine below the detection limit of 10ng/ml). There was no significant correlation between maternal cotinine levels and the reported number of cigarettes smoked by the mother/day ($R^2=0.07$) (Figure 3.1) and between maternal and infant's cotinine ($R^2=0.138$) (Figure 3.2)

There were no significant differences in maternal cotinine, infant's cotinine and the number of cigarettes smoked between S and SM mothers (Table 3.2). There were also no significant differences in maternal cotinine, infant's cotinine and the reported number of cigarettes smoked by the mother between mothers who smoked both tobacco and cannabis and those who tobacco smoked only (Table 3.3).

Analysis of urine samples in SM mothers during antenatal outpatient attendances confirmed that all SM women were substance misusing and misusing a wide range of substances (Table 3.4). Urine drug screen in the postpartum period showed that twelve mothers were positive for methadone, seven were positive for cocaine and four were positive for benzodiazepines (Table 3.5). Three of the twelve mothers positive for methadone misused "non-prescribed" methadone and two mothers with history of cocaine misuse during pregnancy were negative for cocaine on postnatal urinary drug screen. Of the four-benzodiazepine positive mothers, three misused methadone and one was positive for cocaine in addition to the benzodiazepine.

None of the maternal or infants samples tested positive for amphetamines on postnatal urine drug screen.

Table 3.1 Maternal and infant's cotinine levels

Maternal self-reported number of cigarettes smoked/day	7 (2-20)
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Maternal cotinine (ng/ml)	2130 (38-18700)
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Infant cotinine (ng/ml)	171 (10-8760)
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Table 3.2 Cotinine data by maternal substance-misuse status

	Smoking/no substance misuse (n=30)	Smoking/substance misuse (n=19)	P value
Cigarettes/day	10 (2-20)	6(3-20)	0.246
Maternal cotinine (ng/ml)	2330(65.9-5920)	1280 (39.7-18700)	0.552
Infant's cotinine (ng/ml)	450.5(59.5-3300)	172.5 (39.5-8760)	0.496
Birth weight (grams)	2852(1800-3342)	2850 (1824-4384)	0.139

Table 3.3 Cotinine data by maternal cannabis smoking status

	Tobacco Smoking (n=22)	Tobacco and cannabis smoking (n=27)	P value
Cigarettes/day	6 (2-20)	7(3-20)	0.442
Maternal cotinine (ng/ml)	1595 (39.7-8940)	2330 (38.3-18700)	0.124
Infant's cotinine (ng/ml)	92.3 (10-2050)	358.5 (41.7-8760)	0.062

Table 3.4 Results of the antenatal urine analysis of the substance misuse mothers

	Number of subjects	Mean (SD)	Range
Visits to antenatal clinic and urine collected	20	9.33 (6.8)	1-26
Number of occasions a particular substance was detected			
Methadone	13	6.86 (7.14)	0-26
Illicit drug	20	6.95 (4.97)	1-20
>1 illicit drug	17	3.95 (2.99)	1-19
Ecstasy	6	0.90 (1.29)	0-5
Cocaine	13	2.90 (2.84)	0-19
Cannabis	11	4.24 (5.01)	0-18
Morphine	11	2.19 (2.48)	0-20
Any opioid metabolite	11	3.19 (3.68)	0-25
Dihydrocodeine	5	0.52 (0.80)	0-3
Benzodiazepines	6	0.90 (1.29)	0-6

Table 3.5 Methadone and cocaine data

Maternal urine methadone level (ng/ml)	1723.5 (396-2460)
Infant urine methadone level (ng/ml)	350 (16-1934)
Maternal dose of methadone at delivery (mg)	50 (20-100)
Maternal urine cocaine level (ng/ml)	1123 (332-1702)
Infant urine cocaine level (ng/ml)	713.5(81-2952)

Figure3.1 Correlation between maternal cotinine level and the reported number smoked

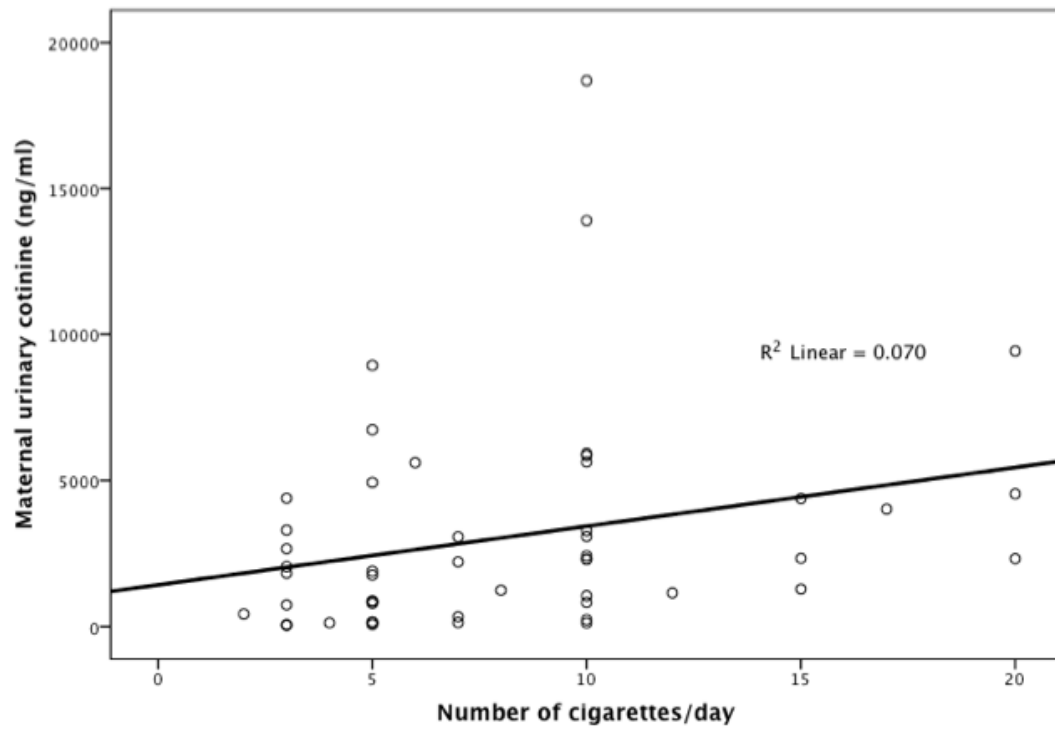
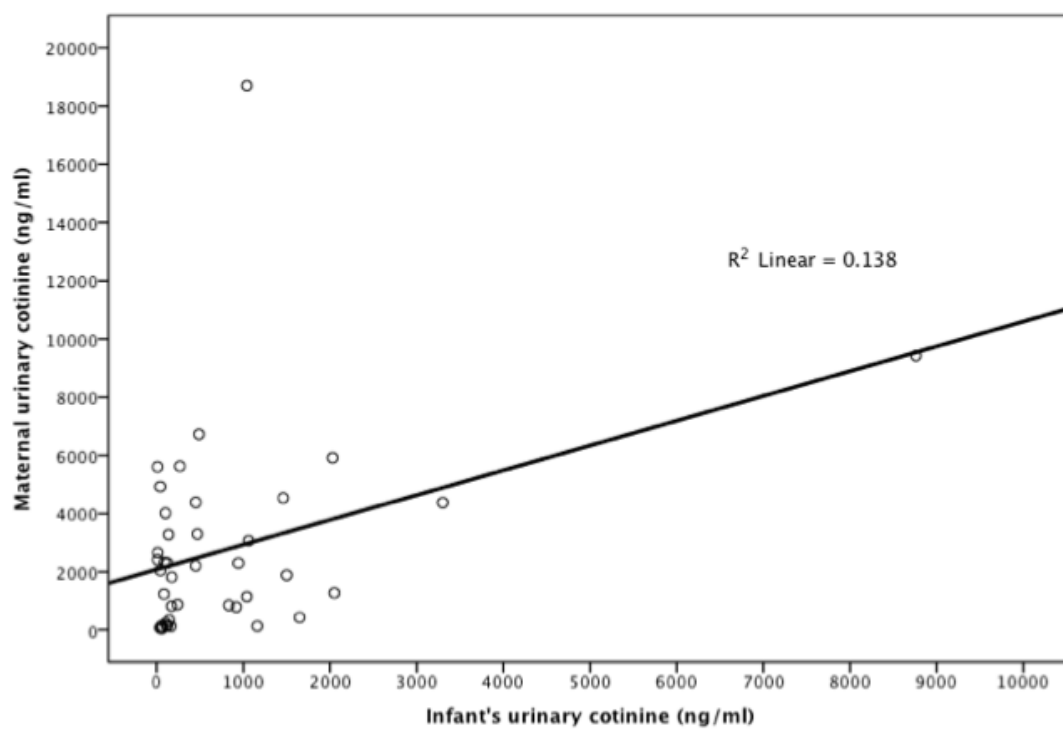


Figure 3.2 Correlation between maternal and infant's cotinine levels



3.4 Discussion

The results demonstrate a lack of correlation between reported maternal smoking and maternal urine cotinine levels obtained in the perinatal period suggesting mothers may not accurately report their smoking habits. On the other hand, all mothers and infants in the control group tested negative for cotinine and illicit drugs, which confirm that mothers in this group did not smoke or substance-misused.

Studies assessing the level of agreement between self-reporting of smoking and biochemical screening for smoking during pregnancy have provided conflicting results. Although some have found a correlation between self-reported smoking and biomarker measures (George et al., 2006, Kvalvik et al., 2012), others have demonstrated significant underreporting by the mothers (Lindqvist et al., 2002, Webb et al., 2003, Parna et al., 2005). In one study that compared the self-reports with serum cotinine in a national sample, 23% of pregnant smokers and 10% of non-pregnant smokers did not disclose their smoking habits (Dietz et al., 2011). In contrast, a recent study reported a high agreement between mothers' self-reported smoking habits during pregnancy and their levels of serum cotinine (Mattsson et al., 2016).

In addition, the results showed no significant correlation between maternal and infant's cotinine levels. There was a time gap between the collections of maternal and infant's urine samples for cotinine analysis. This may explain the lack of correlation between maternal and infant's urinary cotinine levels. Indeed, trans-placental transfer of cotinine from pregnant mothers has been demonstrated in previous studies and was found to increase with advancing gestation (Jauniaux and Gulbis, 2001)

In mothers who substance-misused and smoked, cotinine levels and smoking habits were similar to those who smoked only. Similarly, maternal and infant's cotinine levels did not differ between infants and mothers who smoked both cannabis and tobacco and those who

tobacco smoked only. Over half of the mothers who smoked in the study used tobacco and cannabis. Indeed, the use of cannabis and tobacco (primarily as tobacco cigarettes) frequently co-occurs as described in previous studies (Lynskey et al., 1998), (Degenhardt et al., 2001). In this study, all the mothers who substance-misused also smoked. The rates of nicotine use among abusers of illicit substances and alcohol are reported in the range of 85-100% (Bobo, 1989, Burling and Ziff, 1988) (DiFranza and Guerrera, 1990). Hence nearly three times as many individuals with alcohol and drug problems smoke cigarettes compared to the general population. There is also evidence from previous studies that individuals who substance-misuse tend to be heavy smokers (Hughes, 1995). Continued cigarette smoking has also been linked to worse substance abuse treatment outcomes (Frosch et al., 2000). Hence it is important to determine whether the effects of substance-misuse and smoking are additive on respiratory control and this is an aim of this thesis.

Chapter 4

4 Ventilatory response to hypercarbia in new-borns of smoking and substance misusing mothers

4.1 Introduction

Infants of mothers who smoked and/or substance misused during pregnancy have an increased risk of sudden infant death (SIDS) (Frosch et al., 2000, Ward et al., 1990, Kandall et al., 1993, Hoffman et al., 1988).

A possible explanation for the increased SIDS risk is that affected infants may have brainstem abnormalities which adversely affect their ventilatory control (Lewis and Bosque, 1995) . If that explanation is correct, infants of mothers who smoked and/or substance misused during pregnancy would be predicted to have a reduced ventilatory response to hypercarbia. The aim of this study was to test that hypothesis by assessing the ventilatory response to hypercarbia of infants of substance misusing/smoking mothers, infants of smoking mothers and infants of non-substance misusing, non-smoking mothers. In addition, I tested the hypothesis that smoking and substance misuse would have an additive effect and hence any impairment of the ventilatory response to hypercarbia would be greater in newborns of mothers who both substance misused and smoked compared to those whose mothers only smoked during pregnancy.

4.2 Methods

Infants were eligible for entry into the study if they were born above a gestational age of 36 weeks at King's College Hospital NHS Foundation Trust and had no congenital abnormalities. Informed written parental consent was obtained and the study was approved by the Guys and St Thomas's Hospitals NHS Foundation Trust Research Ethics Committee.

Three groups were recruited:

1. Infants of mothers with a history of substance misuse during pregnancy (SM infants)
2. Infants of mothers with a history of smoking during pregnancy (S infants).
3. Infants of mothers who neither smoked nor misused substances during pregnancy (Controls).

4.2.1 Hypercarbic challenge

The hypercarbic challenge was delivered via a facemask and pneumotachograph through a Custom-made open circuit system with individually adjustable flows of carbon dioxide (CO₂) and air (see Methods chapter 2).

The ventilatory responses to three levels of inspired carbon dioxide (0% (baseline), 2% and 4% CO₂) were assessed

The following outcome measures were assessed:

1. Baseline respiratory rate, tidal volume, minute volume and time to peak tidal expiratory flow
2. The percent change in minute ventilation related to the percent of inspired CO₂
3. The slope of the ventilatory response to hypercarbia, the gradient of the line of best fit of minute volume against inspired CO₂.
4. The mean inspiratory flow (MIF) change: the percent increase from the baseline MIF to the MIF at 4% CO₂.

4.2.2 Assessment of exposure to smoking and substance misuse

In the immediate postpartum period urine samples were obtained from all mothers and infants to assess the profile of maternal substance misuse and for cotinine analysis.

4.2.3 Analysis

Differences between the three groups were tested using the Kruskal Wallis analysis of ranks test for continuous data or the chi-squared test for binary data. Differences in the respiratory results between groups were assessed using regression analysis. Data were transformed using a logarithm or square root transformation to meet regression assumptions. Adjustment was made for baseline differences in birthweight, gestational age, head circumference and gender by fitting the variables as covariates. Results are presented as unadjusted and adjusted arithmetic means (normal data or square root transformed data) or geometric means (log-transformed data). Adjusted means are marginal estimates set to the mean value of the covariates. Analyses were conducted using Stata version 11.

4.2.4 Sample size

The aim was to recruit at least 20 infants in each of the three groups to detect a difference equivalent to one standard deviation in each outcome measurement between the groups with 80% power at the 5% level. A similar magnitude of difference had been detected in the ventilatory response to added dead space between newborns of smoking and non-smoking mothers (Bhat et al., 2005)

4.3 Results

Twenty-two SM, 34 S and 22 control infants were assessed (Table 4.1). The median birth weight of the controls was significantly higher than the SM and S infants ($p=0.015$). The urine analyses demonstrated that all the SM and S mothers had smoked during pregnancy, but none of the mothers of the controls had smoked. There were no significant differences in the proportion of either infants or mothers in the groups with elevated cotinine levels between the S and SM groups (Table 4.2). Analysis of urine samples during antenatal visits confirmed that all SM women were substance misusing. The women were taking cocaine, cannabis, morphine, dihydrocodeine, heroin and/or benzodiazepines. In the SM group, urine analyses in the immediate post-partum period demonstrated five of the twelve women who had been prescribed methadone were also taking it illicitly. Analyses of urine samples in the neonatal period confirmed all SM women had been substance misusing, but that none of the S women or controls had substance misused.

There were significant differences in the mean baseline respiratory rate and minute volume between the groups and these differences remained of similar size and statistical significance after adjustment for baseline neonatal factors. Both the S and SM infants had significantly greater results than the controls (Table 4. 3). The mean time to peak tidal expiratory flow was significantly lower in the SM and S infants than in the controls (Table 4.3). Both the SM and S infants had lower ventilatory responses to 2% ($p<0.001$) and 4% ($p<0.001$) CO₂ than the controls and the differences were not affected by adjustment for neonatal factors (Table 4.3). The ventilatory response to CO₂ was lower in the SM compared to the S infants ($p=0.009$). The mean slope of the ventilatory response was significantly lower in the SM and S infants than the controls ($p<0.0001$) (Table 4. 4) (Figure 4.1). The percentage changes in MIF were significantly lower in the SM and S infants than the controls ($p<0.0001$) (Table 4. 4) and lower in the SM compared to the S infants ($p=0.005$) (Figure 4.2).

Table 4.1 Demographic data demonstrated as median (range) or n (%)

	Control	S	SM	p
N	22	34	22	
Gestational age (wks)	39 (36-42)	39 (36-41)	37 (36-41)	0.12
Birth weight (grams)	3276(2500-4320)	2866(1824-4460)	2844 (1860-3342)	0.015
Head circumference (cm)	34 (32- 37)	34 (30-37)	33 (30-35)	0.048
Gender (male)	14 (63.6%)	22(64.7%)	9 (40.9%)	0.17
Age at study (days)	2 (1-5)	2 (1-9)	2 (1-9)	0.36
Maternal age (years)	31 (20-41)	27 (16-43)	30 (17-40)	0.20

Table 4.2 Urinary cotinine levels in the smoking and substance misuse groups

	S	SM
Maternal urine cotinine		
<10ng/ml	0%	0%
10-500ng/ml	40%	24%
> 500ng/ml	60%	76%
Infant urine cotinine		
<10ng/ml	0%	0%
10-500ng/ml	73%	56%
> 500ng/ml	27%	44%

Table 4.3 Baseline tidal breathing results and the percentage change in minute volume with 2% and 4% inspired CO₂ by maternal smoking and substance misuse status

	Control	S	SM	P values for unadjusted and adjusted group differences
N	22	34	22	
Respiratory rate (breaths/min)¹				
mean (SD ² ; range),	43 (11; 29-73)	49 (12; 36-80)	54 (12; 35-81)	0.006
unadjusted	43 (39, 47)	50 (46, 54)	54 (49, 60)	0.006
mean (95% CI), adjusted ³				
Tidal volume (ml/kg)				
mean (SD ² ; range),	6.9 (1.3; 4.5-9.3)	6.9 (1.5; 4-10)	7 (1.3; 4.9-9.3)	0.41
unadjusted		6.6 (6.1, 7.0)	6.7 (6.1, 7.4)	0.20
mean (95% CI), adjusted ³	7.2 (6.7, 7.8)			
Time to peak tidal expiratory flow (seconds)¹				
mean (SD ² ; range),	0.38 (0.21; 0.17-1.0)	0.27(0.14;0.13-0.68)	0.20 (0.13; 0.09-0.56)	0.0004
unadjusted	0.39 (0.31, 0.48)	0.27 (0.22, 0.33)	0.19 (0.15, 0.24)	0.0002
mean (95% CI), adjusted ³				
Minute Volume (ml/kg/min)¹				
mean (SD ² ; range),	291 (46; 200-400)	342 (75; 220-483)	376 (95; 251-570)	0.0006
unadjusted				0.016
mean (95% CI), adjusted ³	303 (278, 329)	346 (324, 371)	355 (325, 387)	
% change in minute volume at 2% CO₂				
mean (SD ² ; range),	58(26; 8-140)	31 (17; 3-74)	24 (21; 0-75)	<0.0001
unadjusted	59 (50, 69)	31 (23, 38)	23 (14, 33)	<0.0001
mean (95% CI), adjusted ³				
% change in minute volume at 4% CO₂				
mean (SD ² ; range),	104 (36; 42-180)	65 (29; 18-180)	44 (25; 4-91)	<0.0001
unadjusted			42 (28, 56)	<0.0001
mean (95% CI), adjusted ³	111 (98, 124)	66 (55, 76)		

¹ denotes that all means are geometric means due to skewness of the distribution

² SD: standard deviation on natural scale

³ denotes marginal means adjusted for birthweight, gestational age, head circumference and gender (set to mean values)

Table 4.4 The slope of the ventilatory response and the percentage change in mean inspiratory flow (MIF) to hypercarbia by smoking and substance misuse status

	Controls	S	SM	P values for unadjusted and adjusted group differences
Slope of the ventilatory response (ml/kg/min/%)-1				
N	22	34	22	
Mean (SD ¹ ; range) unadjusted	78 (21; 35-123)	56 (23; 19-113)	40 (21; 3.5-97)	<0.0001
Mean (95% CI) adjusted ²	82 (72, 91)	57 (50, 65)	35 (26, 45)	<0.0001
Percentage change in mean inspiratory flow (MIF)				
N	21	32	22	
Mean (SD ¹ ; range), unadjusted	78 (33; 19-156)	55 (30; 2.4-122)	44 (32; 5.3-110)	<0.0001
Mean, adjusted ³	81	55	41	<0.0001
¹ SD:	Standard deviation	on	natural	scale

² denotes marginal means adjusted for birthweight, gestational age, head circumference and gender (set to mean values)

³ The analysis was conducted using square root transformation, unadjusted and adjusted means are therefore untransformed; 95% CIs cannot be calculated. P values are from the transformed analyses. Adjusted values are marginal means adjusted for birthweight, gestational age, head circumference and gender (set to mean values).

Figure 4.1 Slope of ventilator response to hypercarbia by maternal smoking and substance-misuse status

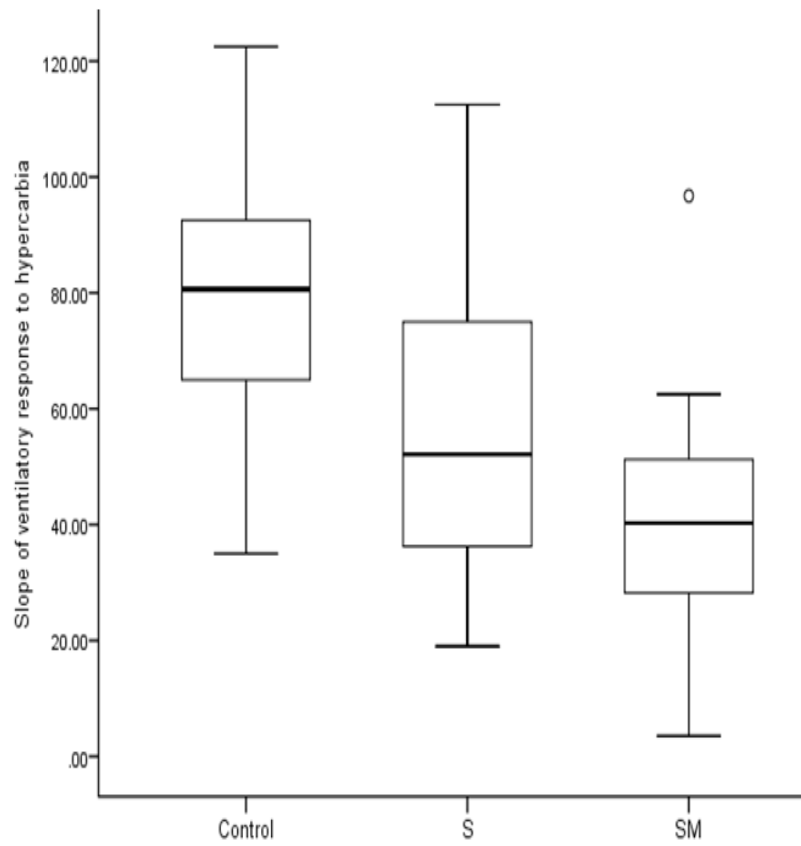
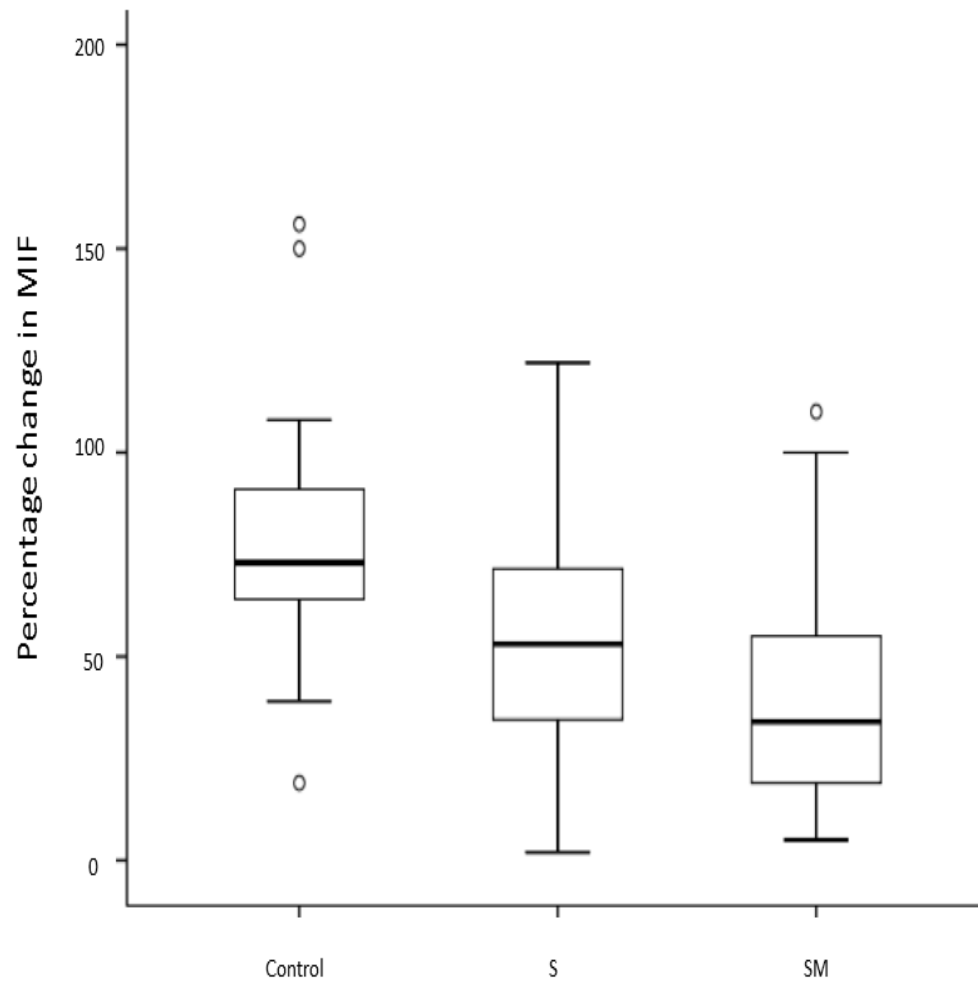


Figure 4.2 Percent change in mean inspiratory flow (MIF) by maternal smoking and substance-misuse status



4.4 Discussion

These results demonstrate that infants of both smoking and substance-misusing/smoking mothers have a dampened ventilatory response to hypercarbia in the immediate newborn period compared to controls; in addition the ventilatory response was poorer in the SM compared to the S infants. There was also lower increase in central respiratory drive, as assessed by the mean inspiratory flow (MIF), in infants of smoking and substance-misusing/smoking mothers compared to the controls. Infants of substance-misusing mothers or smoking mothers have been previously shown to have a reduced carbon dioxide sensitivity (Wingkun et al., 1995) . To my knowledge, this is the first study that has compared the responses to hypercarbia in infants of substance-misuse/smoking mothers and infants of smoking mothers. Infants exposed to methadone in-utero compared to controls have been demonstrated to have decreased sensitivity to a carbon dioxide challenge as measured by the slope of the ventilatory response curve during the first weeks after birth (Olsen and Lees, 1980). The depressed ventilatory response to carbon dioxide lasted on average for 15 days after birth (Olsen and Lees, 1980). It was not possible to assess the effect of methadone per se on the infants' ventilatory responses, as the majority of the mothers from the substance-misuse group were misusing multiple drugs during pregnancy. In addition, as all of the substance misuse mothers smoked, it was not possible to separate out the effects of antenatal substance misuse and smoking. The results, however, suggest that smoking and substance misuse during pregnancy may have an additive adverse effect on the response to hypercarbia. Studies examining infant responses to hypercarbia in relation to maternal smoking have yielded conflicting results (Lewis and Bosque, 1995), (Galland et al., 2003) . In one study no difference was found between the ventilatory and awakening responses to hypercarbia at two to three months of age in infants of smoking mothers compared to controls (Lewis and Bosque, 1995). In another study an increase in the ventilatory response of a combined

hypercarbic/hypoxic challenge in small for gestational infants of smoking mothers compared to controls was demonstrated when the infants were between one to three months of age. In both of those studies (Lewis and Bosque, 1995), (Galland et al., 2003) the infants could have been exposed postnatally as well as antenatally to maternal smoking. In this study infants were studied prior to maternity/neonatal unit discharge and thus were specifically assessed the effect of antenatal exposure to maternal smoking. The results are consistent with the findings from previous study which showed that infants of mothers who smoked antenatally and were studied prior to maternity unit discharge had a dampened ventilatory response to added dead space, in which the major stimulus is hypercarbia when compared to controls (Bhat et al., 2005).

There are strengths and some limitations to this study. Maternal report was not relied on to categorise which group an infant belonged, all the mothers' and infants' urine samples analysed postnatally as well as analysis of the SM mothers' urine samples antenatally. The infants' response to the hypercarbic challenge was assessed in a number of ways and demonstrated consistent findings. There were significant differences in baseline characteristics, but the results remained statistically significant after adjusting for those baseline differences. At baseline, the SM and S infants differed with regard to their respiratory rate, minute volume and time to peak tidal expiratory flow. The differences demonstrated with regard to time to peak tidal expiratory flow are consistent with previous findings, that is a shortened time to peak expiratory flow had been demonstrated in infants of mothers who smoked antenatally compared to controls (Hoo et al., 1998). The higher respiratory rate and minute volume of the SM and S infants at baseline may reflect withdrawal from substances (including nicotine) misused by their mothers. Despite the differences in minute volume at baseline, significant differences in the response to increasing levels of CO₂ were demonstrated between the three groups. The higher baseline minute

volumes of the SM and S infants may explain in part their lower respiratory response to hypercarbia. The controls, however, compared to the SM and S infants achieved significantly higher minute volumes in response to hypercarbia emphasizing that regardless of the baseline differences the SM and S infants had a dampened response.

In conclusion, infants of both smoking and substance-misusing/smoking mothers compared to controls had a dampened response to a hypercarbic challenge in the newborn period. The infants of mothers who substance abused and smoked during pregnancy had a more dampened response than the infants of mothers who only smoked. The results are compatible with dampened chemoreceptor function in infants exposed in utero to smoking and substance misuse. If that abnormality persists beyond the neonatal period, it may explain the increased risk of SIDS in infants of smoking and substance-misusing mothers.

Chapter 5

5 Antenatal substance misuse and smoking and newborn hypoxic challenge response

5.1 Introduction

In utero exposure to illicit drugs such as opioids, marijuana and cocaine is associated with an increased risk of sudden infant death syndrome (SIDS) (Ward et al., 1990). A possible explanation for the increased SIDS risk is that infants of SM or S mothers may have abnormalities of respiratory control. There is a body of evidence demonstrating nicotine exposure could and does influence respiratory control in animal models. For example, prenatal exposure to nicotine may alter the function of peripheral chemoreceptors, as in three-day old rabbit pups exposure to nicotine was associated with reduced dopamine levels and increased expression of tyrosine hydroxylase in the carotid bodies (Holgert et al., 1995). The ventilatory responses to hypoxia has been demonstrated to be attenuated by infusion of nicotine to 7, 17 and 27 day old lambs.(Milerad et al., 1995). Furthermore, nicotine infusion to pregnant rats throughout gestation resulted in the pups having suppressed noradrenergic neuronal activity in the brainstem and forebrain and hyperresponsiveness to hypoxia.(Slotkin et al., 1995). In addition, intermittent hypoxia was demonstrated to result in transient delay in neuronal migration early in the postpartum period in Sprague-Dawley rats, amplified by concurrent nicotine administration.(Zechel et al., 2005) Data from in vivo studies have highlighted that in utero exposure to substance misuse may also affect respiratory control. Infants prenatally exposed to cocaine were demonstrated to have a higher incidence of cardiorespiratory pattern abnormalities than infants with no prenatal drug exposure.(Chasnoff et al., 1989) In another study, full term infants prenatally exposed to cocaine and opiates had less periodic breathing (Silvestri et al., 1991). Even apparently asymptomatic neonates with a maternal history of cocaine use may have degenerative changes or focal infarctions in their basal ganglia (Dogra et al., 1994). Infants of mothers who smoked and/or substance misused during pregnancy may therefore have a poor ventilatory response to hypoxia. The aim of this study was to test that hypothesis by comparing the ventilatory responses to hypoxia of infants

of substance misusing mothers, infants of smoking mothers and infants of non-substance misusing, non-smoking mothers. In addition, a further hypothesis was tested that any change in the ventilatory response to hypoxia would be greater in newborns of mothers who substance misused, as such mothers usually also smoke, compared to those whose mothers had only smoked.

5.2 Methods

Infants were eligible for entry into the study if they were born at 36 weeks of gestational age or greater at King's College Hospital NHS Foundation Trust and had no congenital abnormalities. Informed written parental consent was obtained and the study was approved by the Guy's and St Thomas's Hospitals NHS Foundation Trust Research Ethics Committee.

Three groups were recruited:

1. Infants of mothers who gave on antenatal screening a history of substance misuse during pregnancy (SM infants).
2. Infants of mothers who gave on antenatal screening a history of smoking during pregnancy (S infants).
3. Infants of mothers who neither smoked nor misused substances during pregnancy (controls).

5.2.1 Hypoxic challenge

The hypoxic challenge was delivered via a facemask and custom-made open circuit system using 15% oxygen, as described in Chapter two.

During quiet breathing, the infants were switched from breathing room air to 15% oxygen in nitrogen. The final minute of tidal breathing in air was used as the baseline value. The hypoxic challenge was maintained for five minutes, but terminated if the oxygen saturation level fell below 85%. Infants respond to hypoxia with a biphasic response (Cohen et al., 1997) .

The responses to the hypoxic challenge were determined by:

- 1) The magnitude of increase in minute ventilation from baseline to the peak ventilation
- 2) The magnitude of decline in minute volume from the peak to the lowest minute volume
- 3) The rate of decline in minute volume, calculated as the peak minute volume-lowest minute volume divided by the time from peak to the lowest minute volume.
- 4) The change in the oxygen saturation level from baseline to the lowest oxygen saturation level.

The time to peak minute ventilation was noted; as was the time to the lowest SaO₂ and the baseline end tidal CO₂.

5.2.2 Assessment of exposure to smoking and substance misuse

In the immediate postpartum period, urine samples were obtained from all mothers and infants to assess the profile of maternal substance misuse and for cotinine analysis.

5.2.3 Analysis

Differences between the three groups for continuous data were assessed for statistical significance using multiple regression where possible, with data transformation as needed to meet normality assumptions, if that was not possible the Kruskal-Wallis analysis of ranks test was used. Differences in baseline minute volume, the increase and decrease in minute volume were adjusted for gestational age using multiple regression on the transformed outcomes. The increases in minute volume data were highly skewed and so a square root transformation was required to satisfy Normality assumptions. Since back-transformed confidence intervals are non-interpretable for square root-transformed data, p values are reported using the transformed data for rigour, but means and confidence intervals are presented on the natural scale. For data that could not be transformed, post hoc comparisons were conducted using the Mann Whitney-U test (continuous data) or Chi squared test (categorical data). Where regression analysis was used, post hoc comparisons were made using likelihood ratio tests. Analysis was performed using Stata v11.

5.2.4 Sample size

Recruitment of 20 infants in each of the three groups allowed detection of a difference equivalent to one standard deviation in each outcome between the groups with 80% power at the 5% level. A similar magnitude of difference had been detected in the ventilatory response to added dead space between newborns of smoking and non-smoking mothers.(Bhat et al., 2005)

5.3 Results

Twenty-one substance-misuse (SM), 21 smoking (S) and 19 control infants were assessed (Table 5.1). The mean gestational age of the infants varied significantly between the three groups: SM infants were of significantly lower mean gestational age than the S infants and the controls. Similarly mean birth weight varied significantly between groups, the mean birth weight of the SM infants was lower than that of the S infants and the controls. The urine analysis demonstrated that all the mothers of SM infants were also smoking, but that none of the mothers of the controls were smoking (Table 5.2). The urine analysis also demonstrated none of the mothers of S infants or the controls had been substance misusing. There were no significant differences in the percentage of mothers or infants with cotinine levels in predefined categories between the S and SM groups (Table 5.2). In the SM group, urine analysis in the immediate post-partum period demonstrated five of the 10 women who had been prescribed methadone were also taking it illicitly.

There was significant variability between the groups in mean baseline minute volume, which remained significant after adjustment for gestational age (Table 5.3). The SM infants had a

significantly higher adjusted mean baseline minute volume than controls (395 vs 348; $p=0.005$) (Table 5.3). The initial increase in minute volume during the hypoxic challenge differed significantly between the groups before and after adjustment for gestational age, there was a significantly higher mean increase in the SM group compared to the S group (117 vs 57; $p=0.028$) and the controls ($p=0.034$) (Table 5.3). The magnitude of the subsequent decline in minute volume varied significantly between the groups with a greater magnitude of decline occurring in the SM compared to the controls (220 vs 106; $p<0.001$) and the S infants (220 vs 151; $p=0.018$). The mean rate of decline in minute volume also varied significantly between the groups overall and was greater in the SM infants compared to the controls (4.29 vs 160; $p=0.008$) and the S infants compared to the controls (4.29 vs 3.88; $p=0.011$) (Figure 5.1). There were no significant differences in either the mean baseline oxygen saturation or mean lowest saturation level in response to the hypoxic challenge between the groups (Table 5.4). The mean time to the lowest oxygen saturation, however, was greater in the controls compared to both the infants of S or SM mothers (115, 100, 100 seconds respectively; $p=0.017$) (Table 5.4). There were no significant differences in the mean time of peak minute ventilation or the maximum baseline end tidal CO₂ levels between the three groups (Table 5.4). It was noted, however, that the capnogram results often did not return to zero baseline on inspiration and this may have influenced some of our results.

Table 5.1 Demographics by maternal smoking and substance misuse status

	Controls (n=19)	Smoking (S) (n=21)	Substance misuse (SM) (n=21)	Overall p-value	Post-hoc tests* p- values where applicable
Gestational age (wks)	38.8 (1.7) [39; 36-42]	38.6 (1.7) [39; 36-42]	37.3 (1.6) [37; 36-40]	0.01	S versus control: 0.77 SM versus S: 0.02 SM versus control: 0.007
Birth weight (gms)	3291 (657) [3282; 2500-4972]	3106 (759) [2988; 1860-4384]	2536 (487) [2465; 1730-3192]	0.002	S versus control: 0.41 SM versus S: 0.02 SM versus control: 0.003
Head circumference (cms)	34.2 (1.5) [35; 32-37]	34.3 (1.4) [35; 32-36]	33.6 (0.75) [34; 32-35]	0.15	
Maternal age (yrs.)	30.9 (5.1) [31; 23-38]	27.9 (7.8) [29; 18-43]	30.5 (6.2) [30; 20-40]	0.41	
Gender (male)	58% (11)	71% (15)	43% (9)	0.17	
Mode of delivery (SVD)	23% (11)	40% (19)	36% (17)	0.06	
Age at study (days)	2 (1-5)	2(1-9)	2(1-8)	0.35	

***post-hoc tests only undertaken when the overall comparison of the three groups was statistically significant.**

Table 5.2 Urinary cotinine data by maternal smoking and substance misuse status

	S	SM
Maternal urine cotinine <10ng/ml	0%	0%
10-500ng/ml	40%	23.5%
> 500ng/ml	60%	76.5%
Infant urine cotinine		
<10ng/ml	0%	0%
10-500ng/ml	73%	56%
> 500ng/ml	27%	44%

Table 5.3 Changes in minute ventilation by maternal smoking and substance misuse status

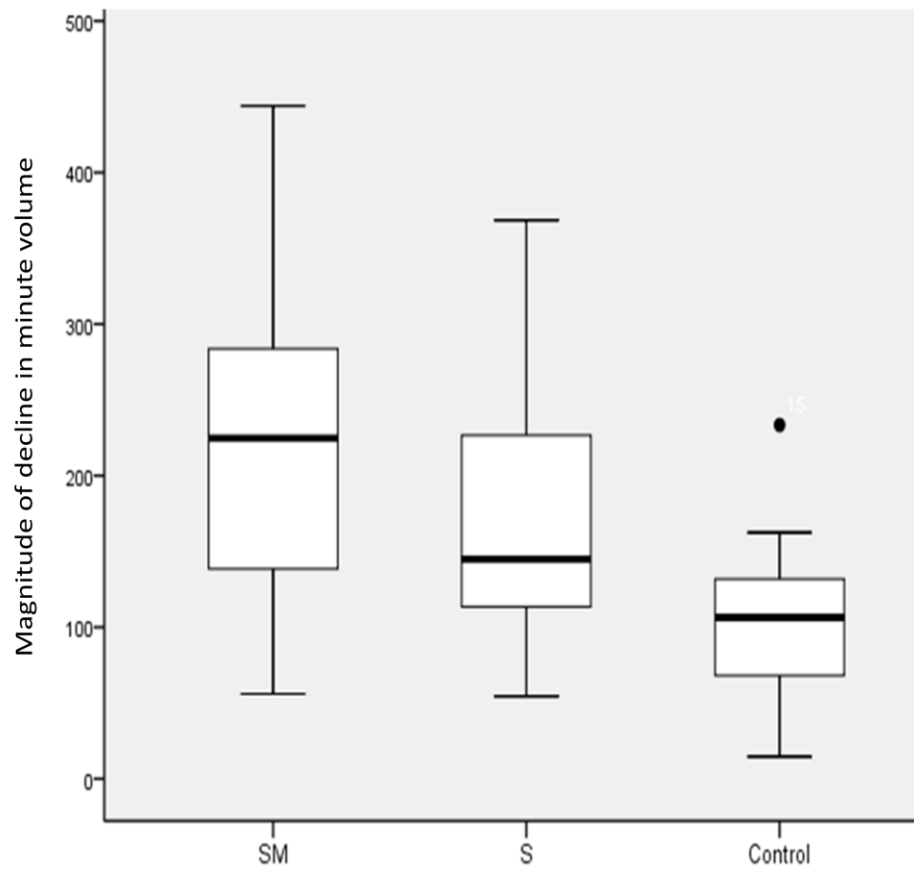
	Unadjusted values				Adjusted for gestational age ¹			
	mean; median (range)				Mean (95% CI)			
	Control n=19	Smoking n= 21	Substance misuse n=21	Over-all P	Control n=19	Smoking (S) n= 21	Substance misuse (SM) n=21	Overall P P for pairs of means
Baseline minute volume (ml/kg/min)	317; 317 (203-405)	354; 344 (253-524)	416; 408 (266-614)	0.003	315 (283, 350)	348 (315, 385)	395 (356, 438)	0.014 S versus control: 0.16 SM versus control: 0.005 SM versus S: 0.10
Increase in minute volume (ml/kg/min)	44; 37 (0-126)	55; 45 (0-163)	121; 89 (0-374)	0.017	47 (13, 81)	57 (25, 89)	117 (83, 150)	0.04 S versus control: >0.99 SM versus control: 0.034 SM versus S: 0.028
Magnitude of decline in minute volume (ml/kg/min)	100; 102 (15-233)	147; 129 (54-243)	230; 225 (56-444)	<0.001	106 (70, 142)	151 (117, 185)	220 (185, 255)	0.0002 S versus control: 0.038 SM versus control: <0.001 SM versus S: 0.018
Rate of decline in minute volume (ml/kg/min)	1.90; 1.70 (0.6-5.0)	7.51;2.58 (0.59-32.17)	7.21 ;4.64 (0.10-31.32)	0.006	1.60 (0.98, 2.60)	3.88 (2.45, 6.12)	4.29 (2.66, 6.92)	0.009 S versus control: 0.011 SM versus control: 0.008 SM versus S: 0.77

¹ Note: adjusted estimates for Increase in minute volume and magnitude of decline in minute volume are calculated from untransformed data as confidence intervals based on square root transformed data cannot be back-transformed. Significance tests are from transformed data

Table 5.4 Oxygen saturation by maternal smoking and substance-misuse status

	Controls (n=19)	Smoking (n=21)	Substance (n=21)	p
Baseline SpO ₂ (%)	99 (96-100)	99 (97-100)	100 (96-100)	0.81
Lowest SpO ₂ (%) with hypoxic challenge	88 (85-94)	88 (85-94)	87 (85-96)	0.65
Time to lowest SpO ₂ (seconds)	115 (60-300)	100 (50-160)	100 (30-200)	0.017
Change in SpO ₂ (%)	10 (5-15)	10 (6-15)	11 (4-15)	0.66
Time to peak minute ventilation (seconds)	94 (10-282)	115 (35-256)	135 (35-286)	0.177
Maximum baseline end tidal CO ₂	35 (27-41)	33 (26-42)	31 (27-38)	0.21

Figure 5.1 Magnitude of decline in minute volume with hypoxia by maternal smoking and substance-misuse status



5.4 Discussion

These results demonstrate that infants of SM mothers had a greater magnitude of decline in ventilation than the controls and S infants and both the SM and S infants had a greater rate of decline in minute ventilation compared to controls. The three groups' gestational ages differed significantly and, as maturity at birth affects respiratory control, adjustment was made for differences in gestational age. Similar significant differences were found after that adjustment. Similarly, differences in the initial increase in minute ventilation between the three groups remained significant after adjustment for gestational age. To my knowledge, this is the first study that has compared the responses to hypoxia in neonates of substance-misuse/smoking mothers and smoking mothers. The results suggest in utero exposure to substance abuse and smoking have an additive effect on the response to a hypoxic challenge and may explain their increase risk of SIDS.

The early excitatory phase of the biphasic ventilatory response to hypoxia is the result of peripheral chemoreceptor stimulation. It is mediated through N-methyl-D-aspartate (NMDA) glutamate receptors in the caudal brainstem. Prematurely born infants with neonatal apnoea were shown to have an increased ventilatory response to hypoxia.(Nock et al., 2004). Reduction in arterial oxygen tension strongly stimulates the carotid bodies, which mediate the rapid increase in ventilation. The authors (Nock et al., 2004), therefore, speculated that previous episodes of apnoea with resultant intermittent hypoxia led to the enhanced peripheral chemoreceptor response to hypoxia. The peripheral chemoreceptors are active from at least 28 weeks of gestation (Rigatto et al., 1975). Fetuses exposed to nicotine and other misused substances are exposed to intermittent/chronic hypoxia in utero which may explain the initial increased ventilatory response to hypoxia observed in this study (Suzuki et al., 1980). The study results demonstrating infants of misusing/smoking mothers had a greater

initial increase in ventilation are supported by the finding that infants who were chronically exposed in utero to opiates had a significantly greater initial ventilatory increase to hypoxia compared to non-opiate exposed controls.(Teichtahl et al., 2005)

Exposure to intermittent chronic hypoxia may explain my findings. Compared to controls, there was a greater rate of decline in the ventilatory response to the SM and S infants and a greater magnitude of decline in the SM infants. In a piglet model, the decline was more marked following exposure to chronic intermittent hypoxia until day ten after birth (Miller et al., 2000) as evidenced by a decline in phrenic electroneurograms (ENGphr). Several neurotransmitters including γ -aminobutyric acid (GABA) (Martin et al., 2004), adenosine (Richter et al., 1999), (Neylon and Marshall, 1991), serotonin (Simakajornboon and Kuptanon, 2005) and opioids (Kato et al., 2000) are involved in the late component of the hypoxic ventilator response. Intracisternal injection of bicuculline, a GABA_A antagonist, in piglets aged two to ten days old reversed the effects of recurrent hypoxia on the ENGphr hypoxic response and eliminated apnoea during hypoxia (Miller et al., 2000). The authors, therefore, speculated that in the newborn period, GABA is released within the brainstem in direct proportion to the number of episodes of recurrent hypoxia, resulting in activation of GABA_A receptors and worsening of hypoxic respiratory depression (Miller et al., 2000). The late phase is mediated in part through platelet derived growth factor (PDGF) receptors maturation of the neuromodulators, particularly NMDA and PDGF- β Receptors mediated pathways occurs during the postnatal period (Simakajornboon and Kuptanon, 2005).

This study has strengths and some limitations. Infants were studied prior to maternity/neonatal unit discharge and thus I was able to assess the effect of only the antenatal exposure to smoking and illicit substances. I assessed substance misuse by analysing samples from the mothers and their infants and cotinine levels were also measured, hence I had an

objective evidence of substance misuse and antenatal smoking. I was unable to assess the effect of substance misuse alone as all the mothers from the substance-misuse group smoked. In addition, I was unable to assess the effect of specific drug misuse as the majority of mothers were misusing multiple drugs. I did not find any significant differences between the groups in the lowest saturation level in response to the hypoxic challenge, but this is likely because the challenge was terminated if the oxygen saturation level fell below 85%. The oxygen saturation level did not fall below 85% in all infants, hence I compared the time to the lowest oxygen saturation level in the three groups. The time taken to reach the lowest SaO_2 was significantly longer in the controls compared to either infants in the S or SM groups, likely reflecting the latter two groups had a greater magnitude of decline in ventilation than the controls. A sidestream method was used to measure ETCO_2 levels using a CO_2 sampling line and capnograph. The sidestream method does not adequately monitor both nasal and oral airflow. When the capnographic waves were examined, it was often noted that capnogram trace did not return to zero baseline on inspiration which may have influenced some of the results. Given the method we used to measure ETCO_2 , it was not possible to evaluate the changes in ETCO_2 at different periods during the hypoxic challenge. The differences in the maximum baseline ETCO_2 levels were not statistically significant, but there was a trend for the S infants to have lower levels than the controls and the SM infants lower levels than the S infants. This trend likely reflects the significantly higher baseline minute volumes in the SM infants compared to the controls and the trend for the SM infants to have higher baseline minute ventilation than the S infants and the S infants to have higher levels compared to the controls. The infants of substance misusing mothers had higher baseline minute ventilation than the other two groups, reflecting they were withdrawing from the substances their mothers had misused. Nevertheless, I was able to show a significant difference in the

response of the SM infants to the hypoxic challenge compared to both the controls and the S infants.

In conclusion, both SM and S infants compared to controls had a greater rate of decline in ventilation as well as a greater increase in ventilation in response to a hypoxic challenge in the newborn period. The results also highlight that SM infants had a greater magnitude of decline in ventilation than the S infants or the controls suggesting that substance misuse and smoking may have an additive effect.

Chapter 6

6 Ventilatory responses to hypercarbia in infants of smoking and substance-misusing mothers at the peak age for SIDS

6.1 Introduction

Sudden infant death syndrome (SIDS) remains a major cause of death in infants less than one year of age in developed countries (Mathews and MacDorman, 2013). Risk factors for SIDS include maternal smoking (Mitchell et al., 1997) and substance misuse (Mitchell et al., 1997) (Blair et al., 1996).

Studies in the neonatal period before maternity unit discharge (chapter 4), demonstrated that infants of smoking and substance-abusing mothers have a dampened ventilatory response to hypercarbia compared to controls. In addition to that, there was an additive effect of substance-misuse since the impairment of the ventilatory response to hypercarbia was more marked in SM infants whose mothers also smoked compared to S infants (chapter 4).

I have, therefore, hypothesized that infants of substance misusing mothers and infants of smoking mothers would have a poorer ventilatory response to hypercarbia compared to infants of non– substance misusing, non-smoking mothers at the peak age of SIDS (post term 6-12 weeks) and that smoking and substance misuse would have an additive effect, such that any impairment of the ventilatory response to hypercarbia would be greater in infants of mothers who both substance misused and smoked. The aims of this study were to test those hypotheses and determine if the dampening of the response was greater in the peak age for SIDS than in the neonatal period.

6.2 Patients and methods

Infants were eligible for entry into this study if they were born with a gestational age of greater than 36 weeks at King's College Hospital NHS Foundation Trust and had been assessed in the neonatal period before maternity unit discharge.

Three groups of infants completed the follow up studies at 6-12 weeks of age:

4. Infants of mothers with a history of substance misuse during pregnancy (SM infants)
5. Infants of mothers with a history of smoking during pregnancy (S infants).
6. Infants of mothers who neither smoked nor misused substances during pregnancy and the postpartum period (controls).

6.2.1 Hypercarbic challenge

Hypercarbic challenge was delivered via a facemask and pneumotachograph through a custom-made open circuit system with individually adjustable flows of carbon dioxide (CO₂) and air. The ventilatory responses to three levels of inspired carbon dioxide (0% (baseline), 2% and 4% CO₂) were assessed (see Methods Chapter 2).

The following outcomes were assessed:

1. Baseline respiratory rate, tidal volume, minute volume and time to peak tidal expiratory flow
2. The percent change in minute ventilation related to the percent of inspired carbon dioxide
3. The slope of the ventilatory response to hypercarbia, this is the gradient of the line of best fit of minute volume against the inspired CO₂ level.

- 4 The mean inspiratory flow (MIF) change was reported as the percentage increase from the baseline MIF to the MIF at 4% CO₂.
- 5 The time constant of the response to hypercarbia, defined as the time taken to achieve 63% of the maximal response to 4% CO₂

6.2.2 Statistical analysis

Differences between in the three groups were assessed for statistical significance using the Kruskal-Wallis one-way analysis of variance or Chi-square as appropriate. Differences between results in the neonatal period and 6-12 weeks of age were assessed for statistical significance using the paired Wilcoxon rank sum test. Analysis was performed using SPSS version 22.0 (SPSS, Inc., Chicago, IL).

6.2.3 Sample size

78 infants were recruited in the neonatal period, with at least 20 infants in each of the three groups. That sample size allowed us to detect a difference between each of the three groups equivalent to one standard measurement with deviation in each outcome of 80% power at the 5% level.

6.3 Results

Forty-four of the 78 infants assessed in the neonatal period completed the follow-up studies at 6-12 weeks of age. There were no significant differences in the gestational age at birth, birth weight, gender and mode of delivery or maternal age between those who did and did not complete the follow-up studies (Table 6.1). Of the 34 infants who did not complete the follow-up studies, some were lost to follow up and others were unable to perform the measurements. Of the 44 infants who completed the follow-up study, there were no significant differences in their weight or age at the time of the study, between the groups (Table 6.2). The cotinine analysis in the neonatal period demonstrated that all the SM mothers and the S mothers were smoking and none of the controls were smoking. In the SM group, eight women were taking prescribed methadone and two cocaine, five mothers misused multiple drugs.

There were no significant differences in the baseline respiratory rate, tidal volume and minute volume between the groups (Table 6.3). The mean time to peak tidal expiratory flow was significantly shorter in the SM and S infants than the controls (Table 6.3). There was an increase in minute volume in response to breathing 2% and 4% CO₂ in all the groups. Both the SM and S infants had a lower ventilatory response to 2% ($p < 0.001$) and 4% ($p < 0.001$) CO₂ than the controls (Table 6.3). The slope of ventilatory response to CO₂ was greater in the controls than the SM and S infants ($p = 0.001$) (Table 6.4) (Figure 6.1) and lower in SM infants compared to S infants ($p = 0.04$). The percentage change in MIF was greater in the controls than the SM or S infants ($p = 0.001$) (Table 6.4) (Figure 6.2) and lower in SM compared to S infants ($p = 0.03$). The time constant of the response to CO₂ was longer in the S and SM infants compared to controls ($p = 0.001$) (Table 6.4) (Figure 6.3) and longer in SM compared to S infants ($p = 0.04$). The percent increases in minute volume at 2% CO₂ ($p = 0.003$) and 4% CO₂ ($p < 0.001$) and the slope of ventilatory response to CO₂ ($p < 0.001$)

were significantly lower at 6-12 weeks of age than in the neonatal period (Table .65, Figure 6.4).

Table 6.1 Comparison of the infant demographics and age of their mothers who did and did not complete the follow up study

	Follow up completed (n=44)	No follow up (n=34)	P value
Gestational age at birth (wks)	39 (36-41)	38.5 (36-42)	0.880
Birth weight (gm)	2852 (1662-4972)	2912 (1650-4112)	0.697
Gender (male)	24 (55%)	24 (69%)	0.238
Spontaneous vaginal delivery	35 (80%)	23 (66%)	0.165
Maternal age (yrs)	29 (18-43)	27 (18-41)	0.127

Table 6.2 Demographic data at follow up by substance misuse and smoking status

N	Control (14)	S (15)	SM (15)	p value
Weight (kg)	4.93 (3.66- 6.68)	4.95 (4-6.5)	4.5 (3.5-5.68)	0.312
Gender (male)	6 (43%)	11(73%)	7 (47%)	0.194
Maternal age (years)	29 (20-37)	30 (20-43)	30 (20-40)	0.878
Age at study (wks.)	8 (6-12)	8 (7-10)	8 (6-10)	0.822

Table 6.3 Baseline tidal breathing results and percentage change in minute volume with 2% and 4% inspired CO₂ by maternal smoking (S) and substance misuse (SM) status

N	Control 14	S 15	SM 15	p value
Respiratory rate, breaths/min	43 (30-70)	44 (26-62)	47 (32-65)	0.524
Tidal volume ml/kg	7 (5-9.4)	7.3 (5.3-9.5)	7(3.3-9.1)	0.861
Minute volume ml/kg/min	280 (200-560)	301 (210-411)	322 (210-415)	0.073
Time to peak tidal expiratory flow (seconds)	0.3 (0.13-0.55)	0.23 (0.11-0.5)	0.19 (0.11-0.39)	0.018
Baseline mean inspiratory flow	10 (6-21)	11 (8-17.7)	11.1 (5.5-17)	0.144
% Change in minute volume at 2% CO ₂	45.9 (19-93.3)	23.6 (10.3-36.7)	16.7 (7.9-45.5)	< 0.001
% Change in minute volume at 4% CO ₂	71.8 (38.9-117.4)	45.7 (31.6-68.5)	37.6 (20.5-70)	<0.001

Table 6.4 The slope of the ventilatory response and the percentage change in mean inspiratory flow to the Hypercarbia by maternal smoking (S) and substance misuse (SM) status

N	Control (14)	S (15)	SM (15)	p value
Slope of the ventilator response (Ml/kg/min/%)	48.3 (22.5-70)	33.7 (24-46.8)	28.8 (18.3-47.8)	0.001
Percentage change in mean inspiratory flow	64.4 (22.2-100)	46.5 (22.2-84.2)	29.6(6.6-60)	0.001
Time constant (seconds)	34.2 (15-52)	54.2 (30-92)	80 (20-160)	0.001

Table 6.5 The ventilatory responses to hypercarbia in the neonatal and 6-12 weeks of age

	Neonatal (n=78)	6-12 weeks (n=44)	p value
% Change in minute volume at 2% CO ₂	41 (2-125)	30 (8-93)	0.003
% Change in minute volume at 4% CO ₂	74 (4-163)	51(20-117)	< 0.001
Slope of ventilatory response ml/kg/min/%	58 (4-123)	37 (18-70)	< 0.001

Figure 6.1 Box and whisker plot of slope of the ventilatory response to hypercarbia by smoking (S) and substance misuse (SM) status

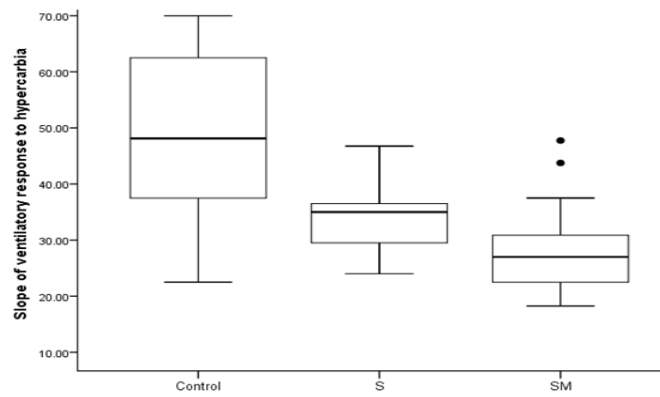


Figure 6.2 Box and whisker plot of the percentage change in mean inspiratory flow (MIF) to hypercarbia by smoking (S) and substance misuse (SM) status

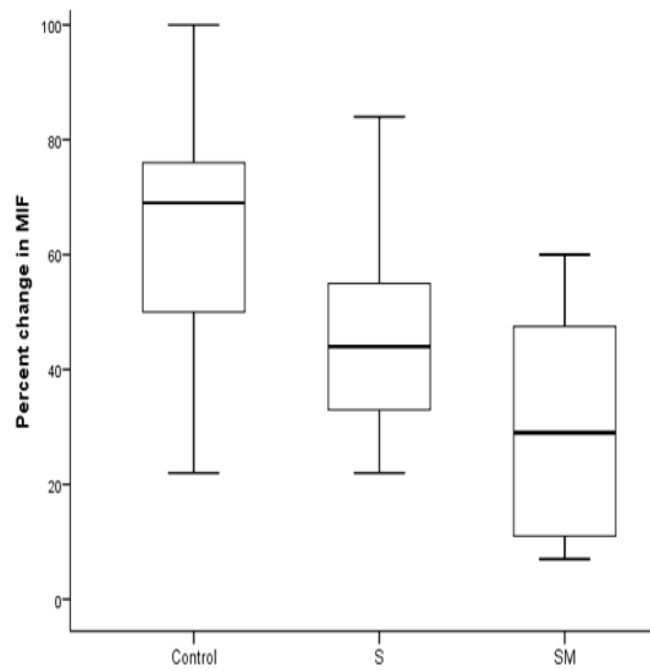


Figure 6.3 Box and whisker plot of the time constant of the response to hypercarbia at 6-12 weeks of age by maternal smoking and substance misuse status

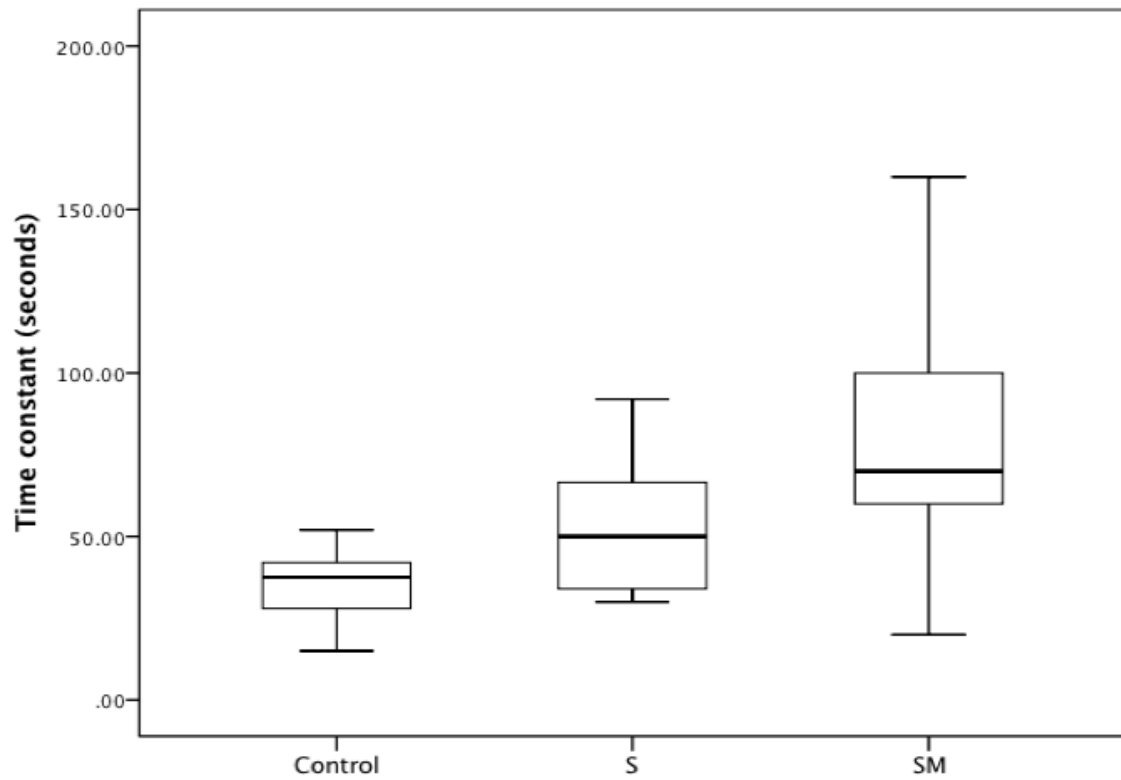
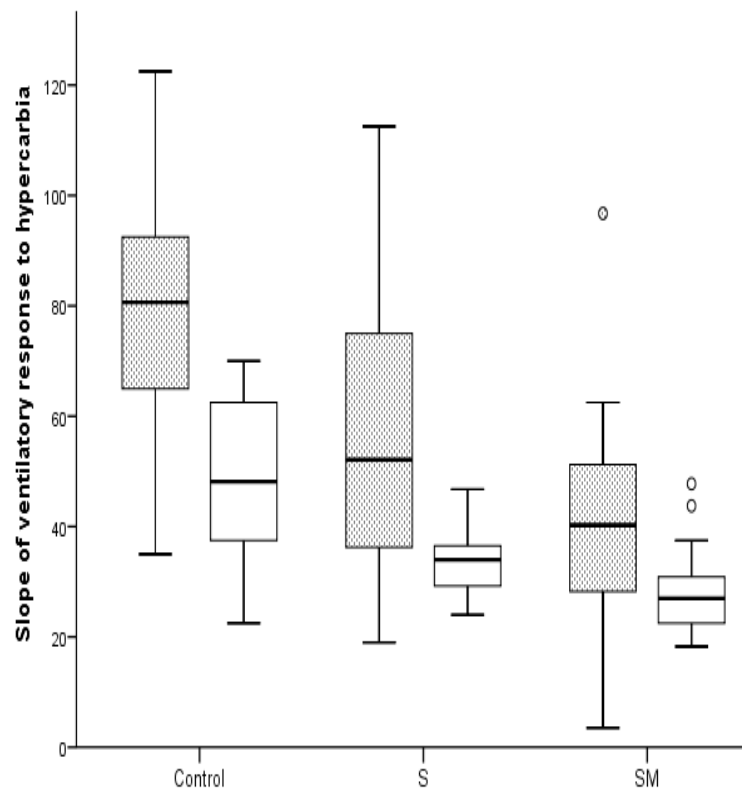


Figure 6.4 Box and whisker plots of the ventilatory response to hypercarbia by smoking (S), substance misuse(SM) and age of study status. Dotted box plots denote neonatal slopes of ventilatory response to hypercarbia.



6.4 Discussion

At the peak age for SIDS these results demonstrated that infants of smoking and substance-misusing/smoking mothers have a dampened ventilatory response to hypercarbia compared to controls. In addition, the ventilatory response to hypercarbia was poorer in the infants of substance misusing mothers compared to those of smoking mothers. There was also a lower increase in central respiratory drive, as indicated by a lower mean inspiratory flow (MIF) and a delayed response to hypercarbia, that is a longer time constant (T_c) in infants of smoking and substance-misusing/smoking mothers. The results also indicate a decline in carbon dioxide sensitivity from the neonatal period to the peak age for SIDS. To my knowledge, this is the first study that has compared responses to hypercarbia between infants of smoking and substance misusing mothers and controls and examined changes in carbon dioxide sensitivity in such infants with increasing age. The results showing a reduction in chemosensitivity at the peak age for SIDS compared to the neonatal period are consistent with previous findings showing the rate of response to added deadspace was significantly dampened with increasing postnatal age (Saiki et al., 2011).

Infants exposed to methadone in utero have been demonstrated to have a decreased sensitivity to a carbon dioxide challenge, that is a lower slope of the ventilatory response curve to CO_2 during the first weeks after birth (Olsen and Lees, 1980). Similarly in infants of substance misusing mothers compared to controls, had lower increased in tidal volume and minute ventilation on exposure to 4% CO_2 in the newborn period (Wingkun et al., 1995). At nine weeks of age, infants of substance misusing mothers were shown to take longer to arouse a hypercapnic challenge (Ward et al., 1992).

Infants of smoking mothers in the newborn period have been shown to have a dampened response to added dead space; the main stimulus during “tube” breathing is hypercarbia (Bhat

et al., 2005). In contrast, infants of smoking mothers studied at one to three months of age did not have a reduced ventilatory response compared to controls (Campbell et al., 2001). In that study, however, infants were exposed simultaneously to both hypoxia and hypercarbia (Campbell et al., 2001).

Serotonin (5 hydroxytryptamine, 5-HT) neurons play a key role in central chemoreception (Richerson, 2004). Prenatal exposures to alcohol and cigarette smoke are associated with harmful effects on the development of the human medullary 5-HT system (Kinney, 2009). Exposure to maternal smoking and cocaine cause upregulation of the 5-HT transporter (5HTT) which is the key regulator of the 5-HT levels at the synapse (Awtry and Werling, 2003, Bauman et al., 2000) (Kelai et al., 2003). Prenatal-perinatal exposure to nicotine alters the activity, electrical properties and central chemosensitivity of the raphe obscurus neurones (Cerpa et al., 2015).

There are strengths and some limitations to the study. The infants' responses to a series of CO₂ challenges were assessed; the order in which the levels were studied was randomised. In all three groups, an increase in the ventilatory response to increasing CO₂ was seen. Longitudinal data were obtained on 44 infants and the paired analysis demonstrated a significantly lower ventilatory response to CO₂ at the peak age for SIDS. It was not possible to assess at follow up all those infants studied in the neonatal period, but there were no significant differences in the demographics of those followed and not followed. Substance misuse and smoking status were confirmed by urinary analyses in the immediate neonatal period. It was not possible to assess the impact of substance misuse alone as all substance-misusing mothers also smoked. The significant differences in the responses of the SM

infants and the S infants, however, suggest that substance misuse and smoking have an additive adverse effect.

In conclusion, infants of smoking and substance misusing/smoking mothers compared to controls had a reduced response to a hypercarbic challenge at the peak age for SIDS, the infants of substance misusing /smoking mothers having the lowest response. These results are compatible with dampened chemoreceptor function in infants exposed to smoking and substance misuse. The response was lower at 6-12 weeks than in the neonatal period and thus I postulate this would increase their vulnerability to SIDS.

Chapter 7

7 Responses to hypoxia in infants of smoking and substance-abusing mothers at the peak age for SIDS

7.1 Introduction

Maternal smoking is associated with at least a three-fold increased risk of SIDS (Mitchell and Milerad, 2006) . Maternal misuse of cocaine (Durand et al., 1990) and opiates (Ward et al., 1990) (Burns et al., 2010) have been shown to increase the risk of SIDS.

SIDS has been hypothesised to be due to brainstem abnormalities that could adversely affect ventilatory control. Studies in the neonatal period (chapter 5), showed that newborns of smoking and substance-abusing mothers had an impaired ventilatory response to hypoxic challenge. Infants of SM mothers had a greater magnitude of decline in ventilation than the controls and S infants and both the SM and S infants had a greater rate of decline in minute ventilation compared to controls (chapter 5).

The aim of this study was to test the hypothesis that infants of substance misusing mothers or smoking mothers would have a poorer ventilatory response to hypoxia compared to infants of non-substance-misusing, non-smoking mothers at the peak age of SIDS. In addition, I hypothesized that smoking and substance misuse would have an additive effect, and hence any impairment of the ventilatory response to hypoxia would be greater in infants of mothers who both substance misused and smoked. A further aim of this study was to determine if any differences between the S, SM and control groups differed at 6-12 weeks compared to the neonatal period.

7.2 Methods

Infants were eligible for entry into the study if they were born at 36 weeks of gestational age or greater at King's College Hospital NHS Foundation Trust and had been assessed in the neonatal period prior to maternity unit discharge. Informed written parental consent was obtained and the study was approved by the Guy's and St Thomas's Hospitals NHS Foundation Trust Research Ethics Committee.

Three groups were recruited:

1. Infants of mothers who gave on antenatal screening a history of substance misuse during pregnancy (SM infants).
2. Infants of mothers who gave a history of smoking during pregnancy and the postpartum period (S infants).
3. Infants of mothers who neither smoked nor misused substances during pregnancy and the postpartum period (controls).

7.2.1 Hypoxic challenge

The hypoxic challenge was delivered via a facemask and custom-made open circuit system using 15% oxygen in balanced nitrogen (BOC Gases, UK) as described in chapter 2.

The responses to the hypoxic challenge were determined by:

- 1) The magnitude of increase in minute ventilation from baseline to the peak ventilation
- 2) The magnitude of decline in minute volume from the peak to the lowest minute volume
- 3) The change in the oxygen saturation level from baseline to the lowest oxygen saturation level.

- 4) Magnitude of increase in heart rate from baseline to peak in response to hypoxic challenge
- 5) Changes in end tidal CO₂ (ETCO₂) with hypoxia

The time to peak minute ventilation was noted, as was the time to the lowest SaO₂.

7.2.2 Statistical analysis

The data were tested using the Kolmogorov-Smirnov test and found to be not normally distributed. Differences between in the three groups were assessed for statistical significance using the Kruskal-Wallis one-way analysis of variance for continuous variables and Chi-square for categorical variables. Differences between results in the neonatal period and at 6-12 weeks post term were assessed for statistical significance using the paired Wilcoxon rank sum test. Analysis was performed using SPSS version 22.0 (SPSS, Inc., Chicago, IL).

7.2.3 Sample size

We recruited 61 infants (21 SM, 21 S and 19 controls) in the neonatal period. That sample size allowed detection of a difference equivalent to one standard deviation in each outcome between the three groups with 80% power at the 5% level. A similar magnitude of difference had been detected in the ventilatory response to added dead space between newborns of smoking and non-smoking mothers (Bhat et al., 2005)

7.3 Results

Thirty-five of the 61 infants were recruited in the neonatal period completed the follow up studies at 6-12 weeks of age. There were no significant differences in gestational age, birth weight, gender, mode of delivery or maternal age between those who did and did not complete the follow up studies (Table 7. 1). Twelve substance-misuse (SM), 12 smoking (S) and 11 control infants were assessed at 6-12 weeks of age (Table 7.2). There were no significant differences between the groups with regard to the weight, gender, age at the study or maternal age.

The urine analysis in the immediate postpartum period demonstrated that all the mothers of SM infants were also smoking, but that none of the mothers of the controls were smoking. The urine analysis also demonstrated that none of the mothers of S infants or the controls had been substance misusing. In the SM group, urine analysis in the immediate postpartum period demonstrated seven women were on methadone, two had misused cocaine and three had misused multiple drugs.

There was no significant difference between the groups in baseline minute volume ($p=0.17$) (Table 7.3). The initial increase in minute volume during the hypoxic challenge did not differ significantly between the groups ($p=0.309$) (Table 7.3). There were no significant differences in the time to peak minute ventilation between the groups ($p=0.056$). The magnitude of the subsequent decline in minute volume varied significantly between the groups ($p=0.02$) with a greater magnitude of decline occurring in the S ($p=0.037$) and the SM infants ($p=0.016$) compared to controls (Table 7. 3) (Figure 7. 1). The baseline ETCO_2 , the ETCO_2 decline and the ETCO_2 increase with hypoxic ventilatory decline did not differ significantly between the groups (Table 7.3).

There were no significant differences in the baseline oxygen saturation or baseline heart rate between the groups (Table 7. 4). The percentage decline in oxygen saturation was significantly different between the groups ($p= 0.031$). The time to the lowest oxygen saturation was greater in the controls than the infants of S ($p= 0.036$) or SM ($p= 0.014$) mothers (Table 7.4). There was a significantly higher increase in heart rate with the hypoxic challenge in the controls compared to S ($p= 0.05$) and SM ($p= 0.01$) infants.

Comparison of the results in the neonatal period and at 6-12 weeks of age showed that the baseline minute volume was significantly higher in the neonatal period ($p=0.009$). There were no significant differences in the magnitude of the increase in ventilation between the two study periods, but the magnitude of decline was significantly higher in the neonatal period compared to 6-12 weeks of age ($p< 0.001$) (Table 7.5) (Figure 7.2).

Table 7.1 Comparison of the infant demographics and maternal age between those who did and did not complete the follow up study

	Follow up completed (n=35)	No follow up (n=26)	P value
Gestational age (wks)	38 (36-40)	39 (36-42)	0.355
Birth weight (gm)	2850 (1730-4970)	3092 (1860-4460)	0.08
Gender (male)	21 (60%)	16 (61.5%)	0.126
Mode of delivery (SVD)	25(71.4%)	18 (69.2%)	0.640
Maternal age (yrs)	28 (18-40)	31 (18-43)	0.233

Table 7.2 Demographics of the infants who completed the follow up studies by smoking and substance misuse status

N	Control (11)	S (12)	SM (12)	P value
Weight (kg)	4.5 (3.66-6.68)	4.5 (4-6.5)	4.36 (3.5-5.6)	0.474
Gender (male)	7 (63.6%)	8 (66.7%)	5 (41.6%)	0.405
Age at study (wks)	8 (6-12)	8 (7-10)	8 (6-11)	0.954
Maternal age (yrs)	30 (20-37)	29 (22-40)	30 (20-40)	0.845

Table 7.3 Changes in minute ventilation and ETCO₂ in response to hypoxia by maternal smoking (S) and substance-misuse status

	Control (n=11)	S (n=12)	SM (n=12)	P value
Baseline minute volume (ml/kg/min)	270 (200-400)	315 (260-475)	325 (220-430)	0.17
Increase in minute volume (ml/kg/min)	60 (20-200)	50 (0-90)	55 (0-120)	0.309
Time to peak minute ventilation (s)	150 (55-310)	80 (50-130)	75 (25-155)	0.056
Decline in minute volume (ml/kg/min)	40 (0-140)	80 (50-140)	100 (40-130)	0.02
Baseline ETCO ₂ (mmHg)	38 (33-46.5)	35 (30-42)	34.5 (32-43.5)	0.23
Decline in ETCO ₂ (mmHg)	3.5 (1-5)	3 (0-8)	3 (2-7)	0.837
Increase in ETCO ₂ (mmHg)	2.5 (1-6.5)	5 (2-6.5)	5 (3-10)	0.052

Table 7.4 Changes in oxygen saturation (SaO₂) and heart rate with hypoxic challenge by maternal smoking and substance misuse status

	Control (n=11)	S (n=12)	SM (n=12)	P value
Baseline SaO ₂ (%)	100 (97-100)	100 (94-100)	99 (97-100)	0.719
Change in SaO ₂ with hypoxia (%)	7 (4-10)	11 (2-16)	10 (5-18)	0.031
Time to lowest SaO ₂ (s)	120 (60-200)	100 (40-180)	90 (40-120)	0.032
SaO ₂ below 85% (n)	0 (0%)	6 (50%)	2 (16.7%)	0.014
Baseline heart rate (beat/min)	125 (90-150)	135 (115-145)	140 (128-150)	0.062
Increase in heart rate with hypoxia (beat/min)	10 (5-30)	6 (0-15)	5 (0-10)	0.033

Table 7.5 Ventilatory response to hypoxia in the neonatal period and 6-12 weeks

	Neonatal (n=61)	6-12 weeks (n=35)	P value
Baseline minute volume (ml/kg/min)	344 (203-614)	300 (200-475)	0.009
Increase in minute volume (ml/kg/min)	47 (0-374)	55 (0-200)	0.221
Decline in minute volume (ml/kg/min)	136 (15-444)	80 (0-140)	<0.001

Figure 7.1 Box and whisker plot of the magnitude of decline in minute volume by maternal smoking (S) and substance-misuse (SM) status

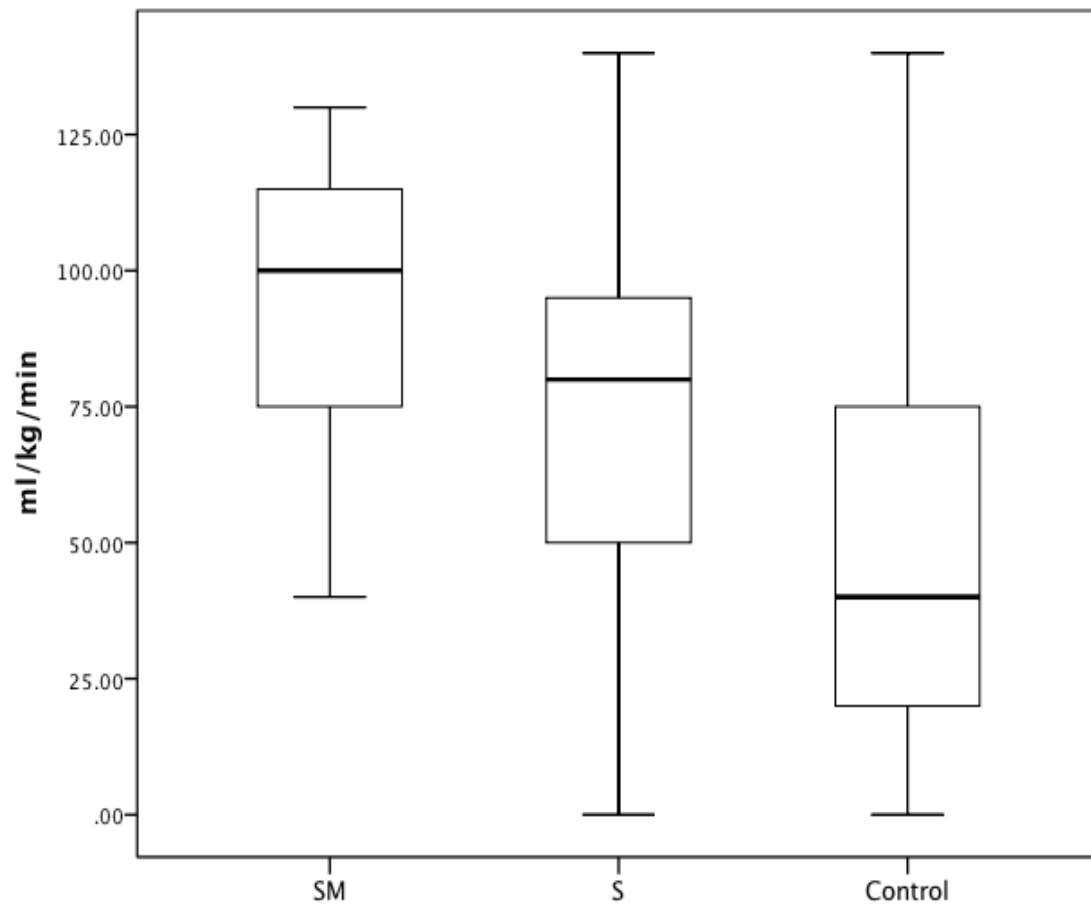
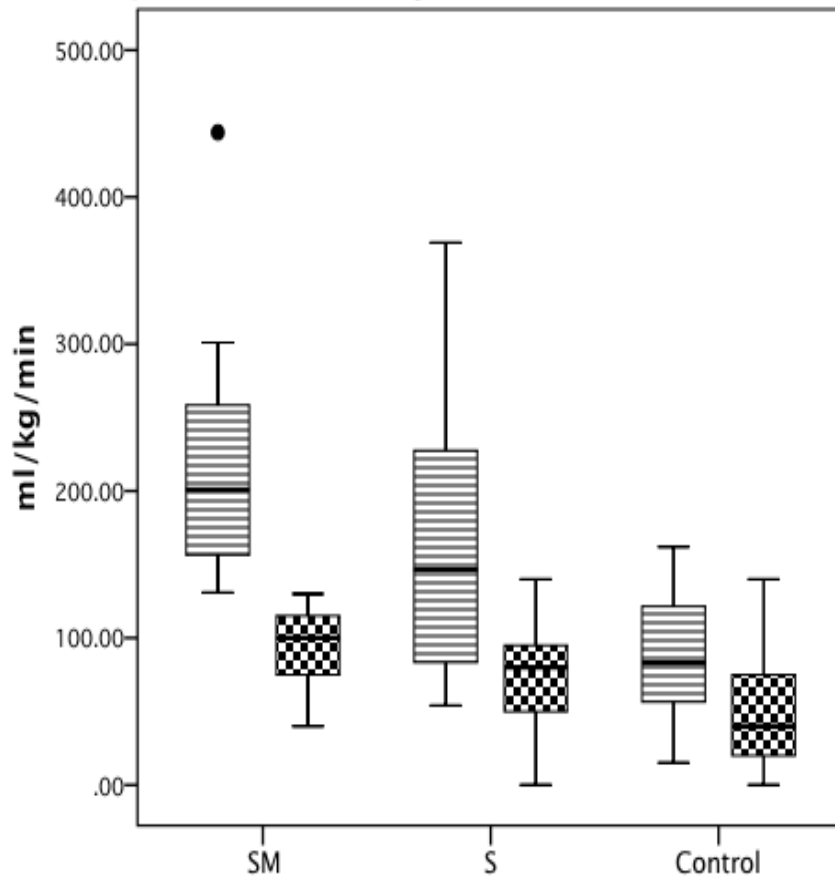


Figure 7.2 Box plot magnitude of hypoxic decline in minute ventilation with hypoxia in the neonatal period and at 6-12 weeks of age

Box plot of the magnitude of decline in minute ventilation with hypoxia by maternal Smoking (S) and substance-misuse (SM) status



Box plots with horizontal lines represents neonatal studies. Dotted box plots represents studies at 2-3 months of age

7.4 Discussion

At the peak age of SIDS infants of SM and S mothers had a greater magnitude of decline in ventilation in response to a hypoxic challenge compared with controls. Importantly, maternal substance misuse and smoking had an additive adverse effect on the response. There was a greater percentage decline in oxygen saturation in response to hypoxia in the SM and S infants compared to controls. In addition, the SM and S infants reached the lowest saturation level earlier in the course of the hypoxic challenge. These effects were greater in the SM infants. Previous studies have highlighted that maternal smoking or substance-misuse impairs the infant ventilatory and arousal responses to a hypoxic challenge out with the neonatal period (Lewis and Bosque, 1995, Ueda et al., 1999). Infants of smoking mothers had a blunted response to a hypoxic stimulus (Ueda et al., 1999, Ward et al., 1992) at 24 months of age. Similarly, at two to three months of age, infants of smoking mothers compared to controls had impaired arousal responses in response to a hypoxic challenge (Lewis and Bosque, 1995). Furthermore, a smaller fall in end-tidal carbon dioxide and impaired arousal responses in response to a hypoxic challenge in infants of substance-abusing mothers at nine weeks of age has been demonstrated (Ward et al., 1992).

The increase in heart rate in response to hypoxia was greater in the controls than the SM or S infants. The ventilatory response to hypoxia increases lung stretch receptor activity via cardiac vagal motoneurons resulting in tachycardia (Dawes et al., 1968, Marshall, 1994, Daly and Kirkman, 1989, Daly et al., 1978). In term born infants aged 2-82 days, maternal smoking affected the magnitude and the time course of heart rate increase in response to hypoxia compared to controls (Sovik et al., 2001). The effect of maternal substance misuse on the cardiac response to a hypoxic challenge has not previously been reported.

One study reported that the biphasic response had disappeared by ten days of age (Brady and Ceruti, 1966), while others have reported that the immature biphasic response to hypoxia persisted into the second month of postnatal life in both prematurely born (Martin et al., 1998) and term born infants (Cohen et al., 1997). Another study showed that the biphasic response to hypoxia persisted in both quiet and active sleep at five to six months of age (Richardson et al., 2007). Our results confirm the persistence of the biphasic response to hypoxia at 6-12 weeks. The magnitude of decline in minute volume in response to hypoxia was less marked at 6-12 weeks of age compared to the neonatal period, reflecting a maturational effect.

The study has strengths and some limitations. The ventilatory response to hypoxia was assessed in three groups whose status was confirmed by urinary analysis in the immediate post-partum period. Although, only approximately half of those recruited in the neonatal period were followed, there were no significant differences in the demographics of those who were and were not followed-up. To my knowledge this is the first study which has compared the responses to hypoxia in infants of SM/S mothers and S mothers both at the peak age for SIDS and in the early neonatal period.

In conclusion, I have demonstrated a change in the ventilatory response to a hypoxic challenge over the first months after birth. Nevertheless, at the peak age for SIDS, there was a greater decline in minute ventilation in response to hypoxia in the infants of smoking mothers and substance misusing mothers and, in particular the infants of substance misusing smoking mothers. This may make them more vulnerable to SIDS.

Chapter 8

8 Discussion

8.1 Summary

The main findings of this thesis are:

- In chapter three, I have shown that there was no correlation between self-reported maternal smoking and maternal urine cotinine levels obtained in the perinatal period.
- In chapter four, I have shown that infants of both smoking and substance-misusing/smoking mothers compared to controls had a dampened response to a hypercarbic challenge in the perinatal period. The infants of mothers who substance abused and smoked during pregnancy had a more dampened response than the infants of mothers who only smoked.
- In chapter five, I demonstrated that infants of SM/S mothers had a greater magnitude of decline in ventilation in response to hypoxic challenge than the S infants or the controls in the newborn period suggesting that substance misuse and smoking may have an additive effect. I also demonstrated that SM and S infants compared to controls had a greater rate of decline in ventilation in response to a hypoxic challenge in the newborn period.
- In chapter six, I have shown that infants of smoking and substance misusing/smoking mothers compared to controls had a reduced response to a hypercarbic challenge at the peak age for SIDS, the infants of substance misusing /smoking mothers having the lowest response. I also demonstrated that the ventilatory response to hypercarbia was lower at 6-12 weeks of age than in the neonatal period.

- In chapter seven, I have shown that at the peak age for SIDS, there was a greater decline in minute ventilation in response to hypoxia in the infants of smoking mothers and substance misusing mothers and, in particular the infants of substance misusing /smoking mothers. I have also shown that there was less decline in minute ventilation in response to hypoxic challenge with increasing postnatal age indicating a maturational effect with increasing age

8.2 Strengths and limitations of the studies:

Maternal reports of smoking and substance misuse practices were not relied on to categorise which group an infant belonged, the mothers' and infants' urine samples were analysed postnatally for cotinine and illicit drugs. Urine samples from mothers and infants in the control group were negative for cotinine and illicit drugs. All mothers in the SM group tested positive for one of five illicit drugs included in the urinary drug screen; with the majority testing positive for more than one drug. In addition, all of the mothers in the SM group also smoked, their urine samples were positive for cotinine. There was no correlation between maternal self-reported number of cigarettes smoked/day and maternal urine cotinine level. Self-reports of smoking and cotinine levels did not differ between infants and mothers in the SM and S groups and between mothers who both tobacco and cannabis smoked compared to mothers who tobacco smoked only. Recent studies assessing the level of agreement between self-reporting of smoking and cotinine have provided conflicting results. Although, some showed a high level of agreement between self-reports and cotinine levels (Mattsson et al., 2016), others did not (Dietz et al., 2011).

The neonatal ventilatory responses to hypercarbia were assessed using a steady state CO₂ inhalation method. This method rather than the added dead space was chosen as it provided a pure hypercarbic challenge rather than the mixed hypercarbic/hypoxic challenge with the added dead space method (Upton et al., 1990). The response to the hypercarbic challenge in the newborn period was assessed between the three groups in a number of ways which demonstrated consistent findings. There were significant differences in baseline characteristics, but the results remained statistically significant after adjusting for those baseline differences. At baseline, the SM and S infants differed with regard to minute volume. Despite the differences in minute volume at baseline, significant differences in the

response to increasing levels of CO₂ were demonstrated between the three groups. To my knowledge, this is the first study that has compared the responses to hypercarbia in infants of substance-misuse/smoking mothers and infants of smoking mothers. Newborns were studied prior to maternity unit discharge, thus the findings of impaired ventilatory response to hypercarbia demonstrated in this thesis reflects the effects of antenatal smoking and substance-misuse. I was unable to study the effects of maternal substance misuse separately as all the SM mothers also smoked. In addition, I was also unable to study the effects of specific drugs on neonatal ventilatory response to hypercarbia because majority of the SM mothers misused multiple drugs.

The newborn's responses to hypoxic challenge were assessed in the three groups of infants (SM, S infants and controls) using 15% oxygen in nitrogen. The responses to hypoxia were assessed by a variety of outcomes; changes in oxygen saturation both in terms of magnitude and time course in response to hypoxia as well as the changes in minute volume at different times during the hypoxic challenge. This thesis has demonstrated that infants of SM mothers had a greater magnitude of decline in ventilation than controls and S infants and both the SM and S infants had a greater rate of decline in minute ventilation compared to controls. The three groups' gestational ages differed significantly and, as maturity at birth affects respiratory control, adjustment was made for differences in gestational age. Significant differences remained after that adjustment. Infants of SM mothers had a greater initial increase in minute ventilation in response to hypoxia compared to S infants and controls. Similarly, differences between the three groups remained significant after adjustment for gestational age. Oxygen saturation level did not fall below 85% in all infants; hence I compared the time to the lowest oxygen saturation level in the three groups. The time taken to reach the lowest SaO₂ was significantly longer in the controls compared to either infants in the S or SM groups, likely reflecting the latter two groups had a greater magnitude of decline

in ventilation than the controls. A sidestream method was used to measure ETCO_2 levels using a CO_2 sampling line and capnography. The sidestream method does not adequately monitor both nasal and oral airflow. Given the method I used to measure ETCO_2 , it was not possible to accurately evaluate the changes in ETCO_2 at different periods during the hypoxic challenge. Hence I did not report the ETCO_2 levels throughout the challenges. Nevertheless, I was able to show a significant difference in the response of the SM infants to the hypoxic challenge compared to both the controls and the S infants. To my knowledge, this is the first study that has compared the responses to hypoxia in newborns of substance-misuse/smoking mothers and smoking mothers. Infants were studied prior to maternity/neonatal unit discharge and thus I was able to assess the effects of only the antenatal exposure to smoking and illicit substances on ventilatory response to hypoxia.

I studied the ventilatory responses to hypercarbia at 6-12 week of age in 44 of the 78 infants studied in the neonatal period. Nevertheless, there were no differences in the demographics between those who did and did not complete the follow up studies. Infants of S and SM mothers have a dampened ventilatory response to hypercarbia compared to controls at the peak age for SIDS. In addition, the ventilatory response to hypercarbia was poorer in the infants of SM mothers compared to those of S mothers. The impaired ventilatory response to hypercarbia in SM and S infants at the peak age for SIDS was demonstrated in different ways; SM and S infants had a lower slope of ventilatory response to hypercarbia, lower magnitude of increase in central respiratory drive in response to hypercarbia and a delay in time response to the hypercarbic challenge compared to controls. I have also demonstrated a decline in CO_2 sensitivity at the peak age for SIDS compared to the neonatal period. There is limited longitudinal data on the changes of chemoreceptor responses with increasing postnatal age. In prematurely born infants studied at PMA of 45 weeks gestation, the rate of

response to added dead space significantly delayed in prone compared to supine position (Saiki et al., 2011).

Responses to hypoxic challenge at the peak age for SIDS demonstrated that infants of SM and S mothers had a greater magnitude of decline in ventilation in response to a hypoxic challenge compared with controls. Importantly, maternal substance misuse and smoking had an additive adverse effect on the response. There was a greater percentage decline in oxygen saturation in response to hypoxia in the SM and S infants compared to controls. In addition, the SM and S infants reached the lowest saturation level earlier in the course of the hypoxic challenge. In response to hypoxic challenge there was a greater increase in heart rate in controls compared to S and SM infants. The effects of maternal substance-misuse on cardiovascular responses to hypoxic challenge have not been previously described. These effects were greater in the SM infants. Comparison of the results of the hypoxic challenge between the neonatal period and 6-12 weeks of age showed that there was a lesser decline in ventilation in response to hypoxia with advancing postnatal age likely reflecting maturation of the hypoxic ventilatory response. There were no differences between the two age groups in the magnitude of initial increase in minute volume in response to hypoxia. Although, only approximately half of those recruited in the neonatal period were followed, there were no significant differences in the demographics of those who were and were not followed-up. To my knowledge this is the first study which has compared the responses to hypoxia in infants of SM/S mothers and S mothers both at the peak age for SIDS and examined changes in ventilatory response to hypoxia SM, S infants and controls with increasing postnatal age.

The hypoxic and hypercarbic challenges were performed during quiet sleep as indicated by observational assessment i.e. when breathing was regular and with less frequent arousals.

This meant that the potential influences of arousal on the ventilatory responses to the

challenges could not be taken into consideration. The hypoxic ventilatory response is dependent on the vigilance state (Henderson-Smart and Cohen, 1988) (Henderson-Smart and Read, 1979) (Lovering et al., 2003, Richardson et al., 2007, Rigatto, 1984, Stephenson et al., 2001). In newborn lambs, active compared to quiet sleep was associated with ribcage deflation during inspiration, a depressed ventilatory response to hypoxaemia despite increases in respiratory rate and delayed arousal (Henderson-Smart and Read, 1979). Similarly, the ventilatory response to hypoxia in adult cats was least in rapid eye movement sleep. (Lovering et al., 2003, Richardson et al., 2007, Rigatto, 1984) A study of male Sprague-Dawley rats demonstrated that ventilation measured by plethysmography was significantly greater during wakefulness than either NREM or REM sleep and ventilation significantly decreased from wakefulness to NREM sleep to REM sleep (Stephenson et al., 2001)

There is conflicting evidence regarding the influence of sleep stage on the ventilatory response to hypercapnia. A difference in resting ventilation and in the response to CO₂ when REM and NREM sleep episodes were compared was not apparent in newborn monkeys less than three weeks of age (Guthrie et al., 1980), nor was it apparent in premature infants (Kalapesi et al., 1981, Rigatto et al., 1980) or term infants until about three months of age (Haddad et al., 1980) . A small increase in ventilation during REM sleep, however, was noted in 11-week-old healthy infants compared to siblings of SIDS victims and infants with a history of apnea (Fagenholz et al., 1976). Although, limiting the studies in this thesis to quiet sleep, may have limited the potential applicability of the studies findings, it would not have influenced the differences detected in ventilatory responses to hypoxia and hypercarbia between the groups.

There is evidence that changes in body temperature alter the ventilatory responses to hypoxia and hypercarbia (Bonora and Gautier, 1989, Cunningham and O'Riordan, 1957, Jennings and Laupacis, 1982, Maskrey and Jennings, 1985) (Maskrey and Nicol, 1979, Vejby-Christensen

and Strange Petersen, 1973). Cats exposed to thermal load had an enhanced ventilatory response to hypoxia (Bonora and Gautier, 1989) and resting, awake dogs had a reduced ventilatory response to CO₂ at lower body temperatures (Jennings and Laupacis, 1982). The environmental temperature at which the studies were undertaken in my thesis were performed was not standardised. Nevertheless, studies in the neonatal period and at 6 to 12 weeks of age were carried out in the same environment; therefore, it seems likely that similar temperatures were used when assessing the ventilatory responses to hypercarbia and hypoxia between the groups.

The hypercarbic and hypoxic challenges were performed in the neonatal period and at 6 to 12 weeks of age using a facemask. There is evidence that the use of a facemask may disturb the normal pattern of respiration. Flemming et al (Fleming et al., 1982) showed that the application of a facemask in term infants, studied at one to four days of age, resulted in a fall in the respiratory rate and a rise in tidal volume in both QS and REM sleep. The authors suggested that the changes in respiratory pattern may have been produced by stimulation of receptors in the trigeminal area (Fleming et al., 1982). Such trigeminal stimulation gives rise to cardiorespiratory reflexes in many newborn animals (Haddad and Mellins, 1977). Others have also shown hypoventilation on face mask application in preterm infants (Chernick and Avery, 1966) and adults (Askanazi et al., 1980). Such changes could also be caused by an increase in airway resistance due to partial airway obstruction (Doershuk et al., 1970). A facemask was used throughout during the hypercarbic and hypoxic challenge in all three groups of infants. Hence, it is unlikely use of a facemask would have influenced the differences detected in the ventilatory responses to hypercarbia and hypoxia between the groups.

8.3 Subsequent research findings:

The results of this thesis have demonstrated effects of antenatal smoking and substance-misuse on newborn's and 6-12 weeks aged infant's chemoreceptor responses and highlighted a possible mechanism in the causation of SIDS. Newborns and 6-12 weeks old infants of smoking and substance-abusing mothers had dampened chemoreceptor responses to hypercarbia and hypoxia in both age groups compared to controls.

SIDS is still the leading cause of post-neonatal infant death in the USA (Kinney and Thach, 2009). Maternal cigarette smoking during pregnancy is the leading risk factor for SIDS. A recent meta-analysis by (Zhang and Wang, 2013) showed that both prenatal and postnatal maternal smoking, were associated with a significantly increased risk of SIDS (OR = 2.25, 95% CI = 2.03–2.50 for prenatal maternal smoking, and OR = 1.97, 95% CI = 1.77–2.19 for postnatal maternal smoking, respectively). In the United-States, although the prevalence of illicit drug use among pregnant women does not appear to have changed since the early 2000s, (Kellogg et al., 2011) the types of drugs that pregnant women are using may have shifted. Chronic medical use of prescription narcotics during pregnancy increased significantly from approximately 2.5 per 1,000 deliveries in 2000 to over 10.0 per 1,000 deliveries in 2008. (Kellogg et al., 2011). This is consistent with the documented increase in therapeutic and nonmedical use of prescription pain relievers in the United States. (Okie, 2010, Manchikanti et al., 2010). A recent large national cohort of nearly 300 centres in the United States, found increases from 2004 through to 2013 in patient admissions, length of stay and resource utilization for infants admitted to NICUs with the neonatal abstinence syndrome (Tolia et al., 2015).

Recent neuropathologic SIDS research focused upon the role of brain regions, particularly the brainstem, that regulate or modulate autonomic and respiratory control during sleep or transitions to waking. The arcuate nucleus is a structure located on the ventral medullary

surface and is thought to be responsible for integration for autonomic, ventilatory and arousal response in addition to having a role in generating a central response to hypercarbia (Kinney et al., 1992); (Kinney et al., 1995). This nucleus has been reported to be hypoplastic and hypomyelinated in SIDS cases (Filiano and Kinney, 1992b); (Lavezzi et al., 2004); (Matturri et al., 2005); (Biondo et al., 2003). In addition, in approximately 50-70% of infants with SIDS, abnormalities involving the serotonergic system have been seen (Paterson et al., 2006); (Kinney et al., 2001). Infants who died of SIDS have been shown to have a higher number and density of 5 HT neurons and a lower density of 5-HT_{1A} receptor binding sites in the regions of medulla involved in cardio-respiratory regulatory function (Paterson et al., 2006). This suggests quite extensive abnormalities involving the serotonin system in the brainstem in SIDS victims involving the synthesis, release and clearance of 5 HT (Moon et al., 2007a). There is emerging evidence that prenatal exposure to maternal cigarette smoking and alcohol affects the postnatal development of human brainstem 5-HT pathways which plays a key role in central chemoreception (Kinney, 2009). In a blinded study of hippocampal morphology in 153 infants with sudden and unexpected death autopsied in the San Diego County medical examiner's office, deaths were classified as unexplained or explained based upon autopsy and scene investigation. Focal granule cell bilamination was present in 41.2 % (47/114) of the unexplained group compared to 7.7 % (3/39) of the explained (control) group ($p < 0.001$) (Kinney et al., 2015). It was associated with a cluster of other dentate developmental abnormalities that reflect defective neuronal proliferation, migration, and/or survival. The authors speculated that the dentate lesions in a large subset of infants with sudden unexplained death might represent a developmental vulnerability that leads to autonomic/respiratory instability and sleep-related death when the infants are challenged with homeostatic stressors. Importantly, these lesions can be recognized in microscopic sections

prepared in current forensic practice and in the future this could be incorporated into the forensic investigations of sudden infant death (Kinney et al., 2015).

Recent animal studies also assessed ventilatory responses to hypoxia following prenatal nicotine exposure. Zhuang et al (Zhuang et al., 2014) recently found that “full term” prenatal nicotinic exposure (fPNE) compared to traditional prenatal nicotinic exposure (tPNE), triggers the apneic response to hypoxia (5% O₂ for 60 min), leading to a lethal ventilatory arrest in rat pups. (Zhuang et al., 2014). The author speculated that nicotinic exposure at the early stage of gestation is critical in generating the delayed hypoxic ventilatory response and subsequent death occurring independently of brain/pulmonary oedema and changes in arterial blood pH/gases (Zhuang et al., 2014)

A study of prematurely born infants at a median post menstrual age of 45 weeks assessed to their response to added dead space in relation to sleeping position however highlighted that the rate of their ventilatory response to added dead space was dampened in the prone compared to supine position (Saiki et al., 2010). This suggests that premature infants mount a poor ventilatory response to an external stress in the prone position and this could possibly make them more susceptible to SIDS.

8.4 Future work:

In this thesis, the effects of maternal smoking and substance misuse on the chemoreceptor responses were studied in the neonatal period and at the peak age for SIDS with studies in both age groups performed in the supine position. Indeed, there is evidence that the risk of SIDS with maternal smoking increases significantly if infants slept in prone position. It will therefore, be valuable to study the combined effects of smoking, substance-misuse and sleeping position in chemoreceptor responses both in the newborn period and at the peak age for SIDS.

Impaired arousal responses to hypoxia and hypercarbia have been hypothesized as important pre terminal events prior to SIDS. Indeed, impaired arousal responses to hypoxia/hypercarbia have been demonstrated in SM infants at the peak age for SIDS (Ward et al., 1992) Similarly, infants of smoking mothers have been shown to have a deficient arousal response to hypoxic challenge at two to three months of age (Lewis and Bosque, 1995). Another important future research study would be to undertake studies in newborns and infants of smoking and substance abusing mothers to assess arousal responses to hypoxia and hypercarbia both in the newborn period and the peak age for SIDS. Serial studies would not only provide valuable information regarding possible mechanisms of SIDS in S and SM infants, but also whether a very high risk group of infants could be identified in the neonatal period and possible interventions assessed.

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